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## Patients with *Chlamydia*-associated arthritis have ocular (trachoma), not genital, serovars of *C trachomatis* in synovial tissue

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### Abstract

Some individuals with a genital *Chlamydia trachomatis* infection develop inflammatory arthritis, but it is unknown whether particular chlamydial serovar(s) engender the disease more often than others. We defined serovar in synovial tissues from arthritis patients infected with this organism. DNA from synovial biopsies of 36 patients with PCR-confirmed synovial *C. trachomatis* was analyzed. Diagnoses included reactive arthritis, undifferentiated oligoarthritis, rheumatoid arthritis, and osteoarthritis. The chlamydial *omp1* and *trpA* genes were amplified, cloned, and 10 or more clones from each sample were sequenced. The cytotoxin locus also was analyzed. *omp1* sequences showed 2 patients having only *C. trachomatis* A serovar, 1 with only B, and 33 having only C, all ocular serovars. Analyses of *trpA* and the cytotoxin locus uniformly displayed standard ocular serovar characteristics for each patient. Identification of ocular chlamydial serovars in the synovia of arthritis patients is unexpected. These observations suggest that urogenital chlamydial infections, while consisting primarily of organisms of genital serovars, include some of ocular serovar(s). They further suggest that during such infections unknown selection pressures favor establishment of the latter in the synovium to the exclusion of genital serovar chlamydiae.

### Keywords

*Chlamydia trachomatis*; inflammatory arthritis; infection; genital infection

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## 1. Introduction

Genital infections with the bacterial pathogen *Chlamydia trachomatis* are a significant problem in the United States, and they must be reported to the Centers for Disease Control (CDC) from all 50 states and the District of Columbia. Data from the CDC indicate that more than 3 million new infections are reported/year, with a stable population of *C. trachomatis*-infected individuals in the US in excess of 6 million [1]. Importantly, genital chlamydial infections can engender serious sequelae, one of which is chronic reactive (inflammatory) arthritis (ReA) [e.g.,2–6]. Reports indicate that ~ 5% of those with a genital chlamydial infection will develop acute ReA, and about half of these latter will proceed to chronicity [e.g.,7]. Further, published data indicate that 20–40% of individuals with inflammatory arthritis but no documented prior genital infection with *C. trachomatis*, and who are given a diagnosis of undifferentiated spondyloarthropathy, oligo- or mono-arthritis, are PCR-positive in synovial tissue and/or fluid for *C. trachomatis* DNA [8,9]. Our studies and those of others have made progress in understanding the molecular and cellular biology of *C. trachomatis*, and in understanding its pathogenic interaction with its host cells in the synovium and elsewhere [6 for review]. However, we do not know whether organisms of one or more particular serovars/strains of the organism are responsible for arthritogenesis, and if so how organisms of that/those serovars initiate and maintain synovial pathogenesis. We also have a poor understanding of why only some individuals develop arthritis following genital chlamydial infection, and why only a portion of those with acute disease progress to chronicity. It is clear that many aspects of disease induction, severity, and chronicity are functions of the serovar of the infecting *Chlamydia*, and of as yet unknown properties of the host genetic background [10,11].

In addition to its role in genital infections, *C. trachomatis* is the etiologic agent for trachoma, a disease that remains the most prevalent cause of treatable blindness worldwide [e.g., 12, 13]. As the designation indicates, serovars of this organism have been differentiated serologically *via* the antigenic structure of the chlamydial major outer membrane protein, encoded by the *omp1* gene [14 and see below]. However, many recent studies have utilized DNA sequence information to identify serovars in clinical or other samples [15; see also below]. Chlamydiae of serovars A, B, Ba, and C are ocular (trachoma) agents, while those of serovars D-K are recognized as standard genital agents. A separate biovar includes the chlamydiae responsible for lymphogranulosum venereum (LGV), designated biovars L1–L3 [14,16]. Importantly, organisms of ocular serovars can cause genital infections, and *vice-versa*, under some conditions [e.g.,17–19]. However, genital infections by ocular serovars are rare [17–19].

Recent reports have demonstrated that ocular and genital chlamydial serovars can be distinguished from one another as groups at several chromosomal loci, in addition to the amino acid or DNA sequence of the *omp1* gene. Organisms of ocular and genital serovars possess different deletions in and around the cytotoxin gene (designated gene Ct166); organisms of ocular serovar B have a large deletion spanning gene Ct162 through the *trp* operon [20]. Further, while genital serovar chlamydiae produce functional products from the *trp* operon, organisms of ocular serovars have deletions in *trpBA* that result in nonfunctional products [21,22]. These differences have been postulated to account in part for tissue tropism and variable pathogenicity between organisms of the ocular and genital serovar groups. A recent study further demonstrated that subtle differences in genomic variation in ocular serovars result in variations in IFN- $\gamma$  sensitivity, growth rate *in vitro*, virulence, and other characteristics [23].

As mentioned, one longstanding question in relation to synovial pathogenesis in *Chlamydia*-associated arthritis concerns whether the disease is elicited by organisms of a particular “arthritogenic” serovar, or whether a range of serovars can disseminate to joints and induce

disease. Further, it is not clear whether those patients who develop only acute ReA following genital chlamydial infection are infected by serovars different from that/those infecting patients who progress to chronic disease. Here, we present surprising observations relating to the infecting *C. trachomatis* serovars identified in the synovia of arthritis patients who are PCR-positive in that tissue for DNA of *C. trachomatis*.

## 2. Results

### 2.1. Infecting chlamydial serovar determined by the DNA sequence of *omp1*

Serovars of *C. trachomatis* often are differentiated and defined on the basis of the predicted amino acid sequence of the *omp1* gene product [e.g., 14,15]. We amplified the complete *omp1* gene, plus some 5' and 3' flanking sequence, using DNA prepared from each of 36 synovial biopsies from individuals known to be PCR-positive for chromosomal DNA from the organism. The resulting DNA sequences were compared to serovar type-sequences in GenBank, and the infecting serovar was deduced from those data. The results shown in Table 1 indicate that of the 36 patient samples so analyzed, 2 were infected with A serovar chlamydiae (patients 7, 12), 1 was infected with organisms of B serovar (patient 36), and the rest were infected with C serovar *C. trachomatis*. None of the patients analyzed showed any DNA sequences characteristic of genital chlamydial serovars in the synovial DNA preparation used as template for PCR amplification. This finding of exclusively ocular rather than genital serovars in these synovial samples from arthritis patients is unlikely to be the result of laboratory contamination, since none of the groups associated with this work studies or has grown or studied ocular chlamydial serovars for many years. Further, negative control PCR assays run with each amplification reaction for each patient sample were universally negative (see also Patients and Methods, below). To be certain that we were not identifying a minor component serovar in a mix with primarily genital serovars, we sequenced at least 10 (and usually 15 or more) clones from each of the 36 patient samples. In all cases, each clone gave a DNA sequence, and thus a deduced serovar, which matched that of the initially sequenced clone from each patient (Figure 1). Moreover, and interestingly, we found relatively little DNA sequence diversity among the clones sequenced from each patient. This is in contrast to data from other laboratories regarding genital samples assessed for chlamydial serovar by DNA sequence [e.g.,<sup>15</sup>; see also below].

### 2.2. Assessment of ocular vs genital chlamydial serovars via the DNA sequence of *trpA*

Studies from other groups have identified DNA sequence characteristics that can differentiate ocular from genital serovars of *C. trachomatis*. For example the *trpA* gene, which encodes the alpha subunit of tryptophan synthase, includes a single base pair (bp) deletion near the 3' end of the coding sequence (nucleotide 528) in organisms of ocular serovars A, Ba, and C, but not in chlamydiae of genital serovars; also, the ocular serovar organisms have a 3 bp deletion upstream of the single bp deletion (nucleotides 408–410) which results in loss of a phenylalanine [22]. These mutations lead to a non-functional product from *trpA*. We PCR-amplified this region of the chlamydial chromosome in selected DNA preparations from the synovial biopsies, then subjected the cloned PCR products to DNA sequencing. As with analyses of the *omp1* gene above, at least 10 (and usually 15 or more) clones from each sample were screened to insure that we were not identifying a minor component of the population. As summarized in Table 1, all of the samples so assessed showed both the triple and single bp deletions at the specified nucleotide locations characteristic of chlamydiae of ocular serovars (Figure 2). Interestingly, patients 15 and 30, both of whom showed C serovar *omp1* sequences exclusively in all clones examined for that gene, gave a mixture of the ocular *trpA* DNA sequence and 3 clones of *trpA* sequence characteristic of the Group I genital serovars (G, F, I, H, J; see [22]). Further, patients 22 and 23, both of whom also had only C serovar *omp1* sequences, gave a mix of ocular *trpA* sequences plus 4 clones of the *trpA* sequence characteristic

of Group II genital serovars (again, see [22]). Additional sequencing of several *omp1* clones from all of these patients failed to yield a sequence characteristic of either Group I or Group II genital serovar organisms, suggesting that in a few infecting organisms the standard C serovar *omp1* sequence is recombined with the genital *trpA* sequence on the same chromosome. This assertion is supported by the DNA sequence of one *trpA* clone from patient 2, which showed a combined ocular type sequence with a Group I genital (J serovar type) sequence on the same molecule (Figure 3). This unusual sequence most probably resulted from a recombination event between an ocular and a genital organism in the same inclusion; such recombination events have recently been described by several groups [e.g.,24,25].

### 2.3. Assessment of the chromosomal deletion at/around the cytotoxin locus

Research from another group has demonstrated that deletions are present in the region upstream of *trpR*, in and around the cytotoxin locus in the plasticity zone on the chlamydial chromosome [20]. On the chromosome of chlamydiae of ocular serovars A, Ba, and C, the deletion is about 5 kbp in length, while in genital serovars D-G, I, and K the deletion is about 5.8 kbp and is shifted somewhat 3'-ward. In organisms of the genital serovars H and J no deletion is present, and in the organisms of ocular B serovar the entire region is missing. We PCR-amplified the region from gene Ct176 to gene Ct179 in selected patient samples and examined the products produced in standard agarose electrophoretic gels. The data summarized in Table 1 indicate that each of the patient samples so analyzed, and for which a PCR product was produced, provided a pattern characteristic of ocular serovars upon analysis in electrophoretic gels. The data in Figure 4 provide representative results for several of the patient samples so analyzed.

### 2.4 Clinical characteristics of the patients analyzed

Individuals with *Chlamydia*-associated arthritis frequently, but not universally, display one or more of panel of extra-articular features, including conjunctivitis, iritis, keratoderma blennorrhagicum, and others [e.g., 4,26]. A question of interest centers on whether the patients whose synovial DNA preparations were studied displayed any particular set or subset of such features that might be reflective of infection with ocular chlamydial serovars. Examination of available clinical data for these patients showed 3 with axial involvement (patients 11, 14, 18), 1 with conjunctivitis (patient 17) and one with retinal inflammation and detachment (patient 4; several others complained of dry eye), and one with psoriasis (patient 3). Thus, the majority of patients studied displayed no ocular involvement, axial manifestations, or skin involvement. Although it is difficult to judge from the somewhat limited available clinical information, our impression is that no meaningful difference in disease severity exists among patients infected with A vs. B vs. C serovar *C. trachomatis*.

## 3. Discussion

In the work presented here, we define the serovars of *C. trachomatis* infecting synovial biopsies from 36 patients with various forms of arthritis, each of whom was known to be PCR-positive in that tissue for this pathogen from our earlier published reports. All patient samples studied were from our large freezer "library" of samples provided for analyses by collaborating clinicians at widely separated geographic locations in the United States. We were expecting to find one or more commonly identified genital serovars of the organism in these samples, but completely unexpectedly we identified only ocular (trachoma) serovars. Also surprisingly, the DNA sequence diversity in the chlamydial *omp1* gene, which is standardly used for serovar definition, was quite low. It is extremely unlikely that these observations result from contamination in the laboratories involved in procurement or processing of the samples, since none of the groups involved grows or studies ocular strains of *C. trachomatis*, and appropriate negative controls were included with each and every assay run. Moreover, processing and

analyses of samples have always taken place in several widely separated laboratories under carefully controlled conditions.

Whether, and if so by what means, chlamydiae of the A, B, and C serovars are in fact uniquely arthritogenic as opposed to those of genital serovars remains to be established. One possible explanation for the apparently exclusive presence of organisms of ocular serovars in the synovia of arthritis patients may be that these organisms simply disseminate from the site of primary infection more efficiently than do all/most genital serovar chlamydiae. Most genital infections with *C. trachomatis* probably are not clonal, *i.e.*, comprised of a single serovar of the organism. Thus, alternatively and along the same line, it may be that both ocular and genital serovars disseminate equally well from the infected genital tract, but that ocular serovars are positively selected from the infection mix by some as yet unknown means once the organisms reach the synovium. Further, some observations suggest that ocular chlamydial strains transit to the persistent state more easily than do genital strains (Dr. G.I. Byrne, personal communication). It is chlamydiae in this latter infection state that are responsible for inflammatory arthritis, and it may be the case that rapid transition to persistence gives *C. trachomatis* of the ocular strains some advantage in establishing long-term residence in the synovium. Much more study will be required to examine these and other possibilities.

Regardless, one issue that the data presented here probably does explain at least in part relates to the epidemiology of *Chlamydia*-induced arthritis. That is, as mentioned above it has never been clear why only a small proportion of individuals who acquire a documented genital chlamydial infection develop the acute inflammatory arthritis. Studies from many laboratories have shown that, while ocular serovars can and do cause genital infections, those serovars are only rarely identified in epidemiologic studies of genital chlamydial infection [*e.g.*, 27–29]. Thus, the low percentage of infected patients who develop acute arthritis may reflect little more than the rarity of ocular serovars in the overall pool of organisms involved in causing genital infections. It is of interest to note here as well that ocular features, such as uveitis, iritis, and conjunctivitis, are sometimes identified as extra-articular features of patients with *Chlamydia*-associated arthritis [*e.g.*, 4, 26]. The presence of chlamydiae of ocular serovars in disseminated sites in such patients may be in part responsible for those ocular effects, although only one of the patients whose DNA was studied here had conjunctivitis. More study will be required to define this issue.

An issue these serovar data do not help to explain is why only about half the number of individuals who develop acute arthritis following a documented genital chlamydial infection progress to chronic disease. The patient samples selected for analysis from our freezer “library” included those with acute and others with chronic arthritis, since we have been interested in the details of host-pathogen interaction in both disease contexts [*e.g.*, 6, 30–32]. The patients studied with acute disease at the time of sample procurement (*e.g.*, patients 1, 13, 15, 28; see also below) displayed no difference in serovar type of infecting organisms compared to the serovar type chlamydiae identified in patients with chronic disease. Thus while more study will be required, our observations provide no evidence for differential serovar infection in patients who develop only acute arthritis *vs.* those who progress to chronic disease. Further, while detailed clinical assessments are not available at this point for each of the patients studied here, our impression is that disease severity is roughly equivalent in patients infected with each of the ocular serovars identified, as judged from relevant information that is available.

As mentioned above, the samples examined here for serovar were obtained from different, and rather widely separated, regions of the United States, indicating that arthritis presumably due to the synovial presence or organisms of ocular chlamydial strains is not confined to a single geographical region. Those locations are, however, in the eastern USA, and it therefore will be of interest to obtain and analyze samples from patients at clinics located on the west coast

and elsewhere. Readers will note that we purposely chose samples from patients known to be PCR-positive for chromosomal DNA but without regard to specific diagnosis; that is, the patients studied here had various forms of arthritis, including but not limited to reactive arthritis due to chlamydial infection. We do not mean to contend that osteoarthritis is caused by chlamydial infection of the joint, of course; rather, we previously demonstrated that 4–5% of patients with that disease, and about the same percentage of perfectly normal control individuals as well, have *C. trachomatis* DNA in synovial tissues [33]. Moreover, synovial PCR-positivity for the organism in patients with rheumatoid arthritis does not indicate that the infecting organism caused the disease; we are, however, re-examining the possibility that chlamydiae do contribute to genesis of this disease both in the laboratory and in an extensive literature review [34; see also 35]. Our point in assessing the serovars of *C. trachomatis* found in synovial samples from PCR-positive patients with various arthritides was to determine if one or more specific serovar(s) is/are uniquely responsible for genesis of *Chlamydia*-induced ReA. In the sense that only ocular serovar chlamydiae are found in the synovium of the patients studied, this does appear to be the case.

It is the case that organisms of C serovar are strongly predominant in samples from the patients studied, and it is not at all clear why chlamydiae of this trachoma serovar are so prevalent in synovial materials. In trachoma studies, C serovar organisms have been identified commonly in isolated indigenous communities in western Australia, in Nepal, and elsewhere [e.g., 36–38]. As indicated above, this serovar indeed has been identified in genital samples, although its occurrence in genital contexts is relatively rare [e.g., 27–29]. Our data indicate that chlamydial infections of the urogenital system are rarely if ever clonal, and that some inocula include a small number of C serovar organisms, or more rarely A and/or B serovar organisms; a selection process favoring those serovars must take place at either the level of dissemination from the genital system or at the level of establishment of stable maintenance of infection in the joint. It is not clear what variations in the C serovar genome, as opposed to the genomes of A or B serovar, enable it to engender inflammatory reactive arthritis in a majority of patients. These are issues that we are investigating.

## 4. Patients and Methods

### 4.1. Patients and diagnoses

DNA preparations from synovial tissue samples from 36 patients were chosen from those available in our freezer “library” of samples. Each sample was chosen on the basis of adequate quality and amount of DNA available for the analyses planned, and because each sample had been demonstrated to be PCR-positive for DNA sequences on the chromosome of the organism in various previous studies, using published, well-characterized screening primer systems [e.g., 30,31]. All samples described here were procured under approved protocols using the standard Parker-Pearson technique [39]. Samples were immediately frozen at –80°C and shipped on dry ice to the Hudson laboratory for analyses. The general characteristics of the 36 patients from whom samples were procured are summarized in Table 1; additional, more detailed clinical information for some patients is not available, since some samples were procured for study as long as 20 yr ago.

The diagnosis for each patient was made according to criteria of the American College of Rheumatology or elsewhere [see below]. ReA occurs 1–6 wk after the initial genital chlamydial infection; clinical symptoms can include inflammatory arthritis, iritis, urethritis, and a characteristic rash on the palms and soles or penis. Most patients have only some of these organ systems involved. These diagnostic criteria require a peripheral arthritis occurring in association with urethritis or cervicitis [26]. The Third International Workshop on ReA requires a peripheral arthritis with sacroiliac involvement and a preceding gastrointestinal or genitourinary infection [40]. The current ACR definition might be too limited in scope, and

the latter's reliance on a preceding infection could lead to under-diagnosis, particularly since asymptomatic chlamydial infections are common [9]. The term undifferentiated oligoarthritis (UO) is non-specific and refers to an inflammatory arthritis involving 2–4 joints. Patients often present in such a manner with no other associated features or diagnostic testing, thereby making a definitive diagnosis difficult. Over time, many of these patients develop features making the correct diagnosis more clinically apparent. Data exist suggesting that *C. trachomatis*, and the other causative organisms of ReA, can cause an incomplete clinical picture resulting in a diagnosis of UO [8,41].

#### 4.2. Analyses

Total DNA had been prepared from each synovial tissue sample using the hot phenol method extensively described by us [30,31]. Prior to performing the present analyses, the quality of each such preparation was assessed by PCR targeting the host actin gene [30,31], and the overall amount of DNA available for analyses was assessed spectrophotometrically. The *omp1* gene, encoding the *C. trachomatis* major outer membrane protein, was amplified from each patient DNA preparation using a high fidelity polymerase (Platinum *Taq*<sup>TM</sup>, Invitrogen, Carlsbad, CA USA), and the primers 5'-ggacatctgtctggcttaact-3'/5' + 5'-gcgctcaagtagaccgatatagta-3' (outer), and 5'-ggacgcagtgccgcagaaaag-3'/5' + 5'-gataagcttactaagaaaagatcc-3' (inner) [42]. The relevant segment of the chlamydial *trpA* gene was similarly prepared from DNA preparations of selected samples using the primers 5'-atgatcgggcttagcagaa-3' + 5'-ttgtgcaagtgcagtcaga-3' (outer) and 5'-cagcacctttatcacacgga-3' + 5'-tcacaatgaaacctatgaaat-3' (inner); the outer primer set was designed by us using GeneRunner<sup>TM</sup> software (Hastings Software, Westwood NJ USA), and the inner primer set is from Caldwell *et al.* [22]. The various PCR products were cleaned using the Qia-Quick<sup>TM</sup> PCR Purification Kit (Qiagen, Valencia, CA USA), then cloned into the pGEM-Teasy<sup>TM</sup> vector, using conditions specified by the manufacturer (Promega, Madison, WI USA; see also [31]). DNA sequencing was done at the core facility at Wayne State University, and at least 10 clones from each patient and for each gene target were sequenced. Serovar determinations from those sequences were done by BLAST search of the *omp1* sequences obtained and computer-based comparison to known serovar-specific sequences. Analysis of the deletions in the *trpA* gene was done by comparison to data given in ref. [22]. Analysis of the large chromosomal deletion in the region of the *toxB* gene was done by PCR amplification of sequences flanking the deletion in selected samples using the primers 5'-agaaacttgatcggatcgggtaa-3' + 5'-ccttgcatggacggtgatataca-3' and 5'-gagctgcaatctatgaagcaagctc-3' + 5'-gctccatagcaccttaaatgccc-3'. This primer set was designed by us using GeneRunner<sup>TM</sup> software. Amplification products were displayed on standard 1% agarose electrophoretic gels [*e.g.*, 31]. In some samples, no amplification product was produced after several repeats of the assay (see Table 1); the lack of product in these cases is most probably due to alterations in DNA sequence at the site(s) of primer annealing, thus obviating primer binding. Cycling conditions for each of the PCR reactions used are available upon request [see also 42].

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18 mkkllkslv faalssassl qalpvgnpae pslmidgilw egfggdpcdp cttwcdaism rvgyygdvfv drvlktdv**D**k efqmgaa~~ptt~~ sdvaglqndp ttnvarpnpa  
 11 mkkllkslv faalssassl qalpvgnpae pslmidgilw egfggdpcdp cttwcdaism rvgyygdvfv drvlktdv**n**k efqmgaa~~ptt~~ sdvaglqndp ttnvarpnpa  
 15 mkkllkslv faalssassl qalpvgnpae pslmidgilw egfggdpcdp cttwcdaism rvgyygdvfv drvlktdv**n**k efqmgaa~~ptt~~ sdvaglqndp ttnvarpnpa  
 13 mkkllkslv faal**R**sassl qalpvgnpae pslmidgilw egfggdpcdp cttwcdaism rvgyygdvfv drvlktdv**n**k efqmgaa~~ptt~~ sdvaglqndp ttnvarpnpa  
 C mkkllkslv faalssassl qalpvgnpae pslmidgilw egfggdpcdp cttwcdaism rvgyygdvfv drvlktdv**n**k efqmgaa~~ptt~~ sdvaglqndp ttnvarpnpa  
  
 18 ygk~~h~~m~~q~~daem ftnaaymaln iwd~~r~~f~~d~~v~~f~~ct lgattgylkg nsasfnlvgl **S**gtktq~~s~~ss~~f~~ ntaklipnta lneavvelyi nttfawsvga raalwecgca tlgasf~~q~~yaq  
 11 ygk~~h~~m~~q~~daem ftnaaymaln iwd~~r~~f~~d~~v~~f~~ct lgattgylkg nsasfnlvgl **f**gtktq~~s~~ss~~f~~ ntaklipnta lneavvelyi nttfawsvga raalwecgca tlgasf~~q~~yaq  
 13 ygk~~h~~m~~q~~daem ftnaaymaln iwd~~r~~f~~d~~v~~f~~ct lgattgylkg nsasfnlvgl **f**gtktq~~s~~ss~~f~~ ntaklipnta lneavvelyi nttfawsvga raalwecgca tlgasf~~q~~yaq  
 13 ygk~~h~~m~~q~~daem ftnaaymaln iwd~~r~~f~~d~~v~~f~~ct lgattgylkg nsasfnlvgl **f**gtktq~~s~~ss~~f~~ ntaklipnta lneavvelyi nttfawsvga raalwecgca tlgasf~~q~~yaq  
 C ygk~~h~~m~~q~~daem ftnaaymaln iwd~~r~~f~~d~~v~~f~~ct lgattgylkg nsasfnlvgl **f**gtktq~~s~~ss~~f~~ ntaklipnta lneavvelyi nttfawsvga raalwecgca tlgasf~~q~~yaq  
  
 18 skpkveel~~n~~ lcnaseftin kpkgyvgaef plnitagtea atgkdasid yhewqaslal s**C**rlnmftpy igvkw~~s~~r~~v~~s~~f~~ dadtiriaqp klaeaildvt tlnptiagkg  
 11 skpkveel~~n~~ lcnaseftin kpk**E**yvgaef plnitagtea atgkdasid yhewqaslal syrlnmftpy igvkw~~s~~r~~v~~s~~f~~ dadtiriaqp klaeaildvt tlnptiagkg  
 13 skpkveel~~n~~ lcnaseftin kpkgyvgaef plnitagtea atgkdasid yhewqaslal syrlnmftpy igvkw~~s~~r~~v~~s~~f~~ dadtiriaqp klaeaildvt tlnptiagkg  
 13 skpkveel~~n~~ lcnaseftin kpkgyvgaef plnitagtea atgkdasid yhewqaslal syrlnmftpy igvkw~~s~~r~~v~~s~~f~~ dadtiriaqp klaeaildvt tlnptiagkg  
 C skpkveel~~n~~ lcnaseftin kpkgyvgaef plnitagtea atgkdasid yhewqaslal syrlnmftpy igvkw~~s~~r~~v~~s~~f~~ dadtiriaqp klaeaildvt tlnptiagkg  
  
 18 svvsagtdne ladt~~m~~qivsl qlnkmksrks cgiavgttiv dadkyavtve arlideraah vnaqfrf  
 11 svvsagtdne ladt~~m~~qivsl qlnkmksrks cgiavgttiv dadkyavtve arlideraah vnaqfrf  
 13 svvsagtdne ladt~~m~~qivsl qlnkmksrks cgiavgttiv dadkyavtve arlideraah vnaqfrf  
 13 svvsagtdne ladt~~m~~qivsl qlnkmksrks cgiavgttiv dadkyavtve arlideraah vnaqfrf  
 C svvsagtdne ladt~~m~~qivsl qlnkmksrks cgiavgttiv dadkyavtve arlideraah vnaqfrf

**Figure 1.**

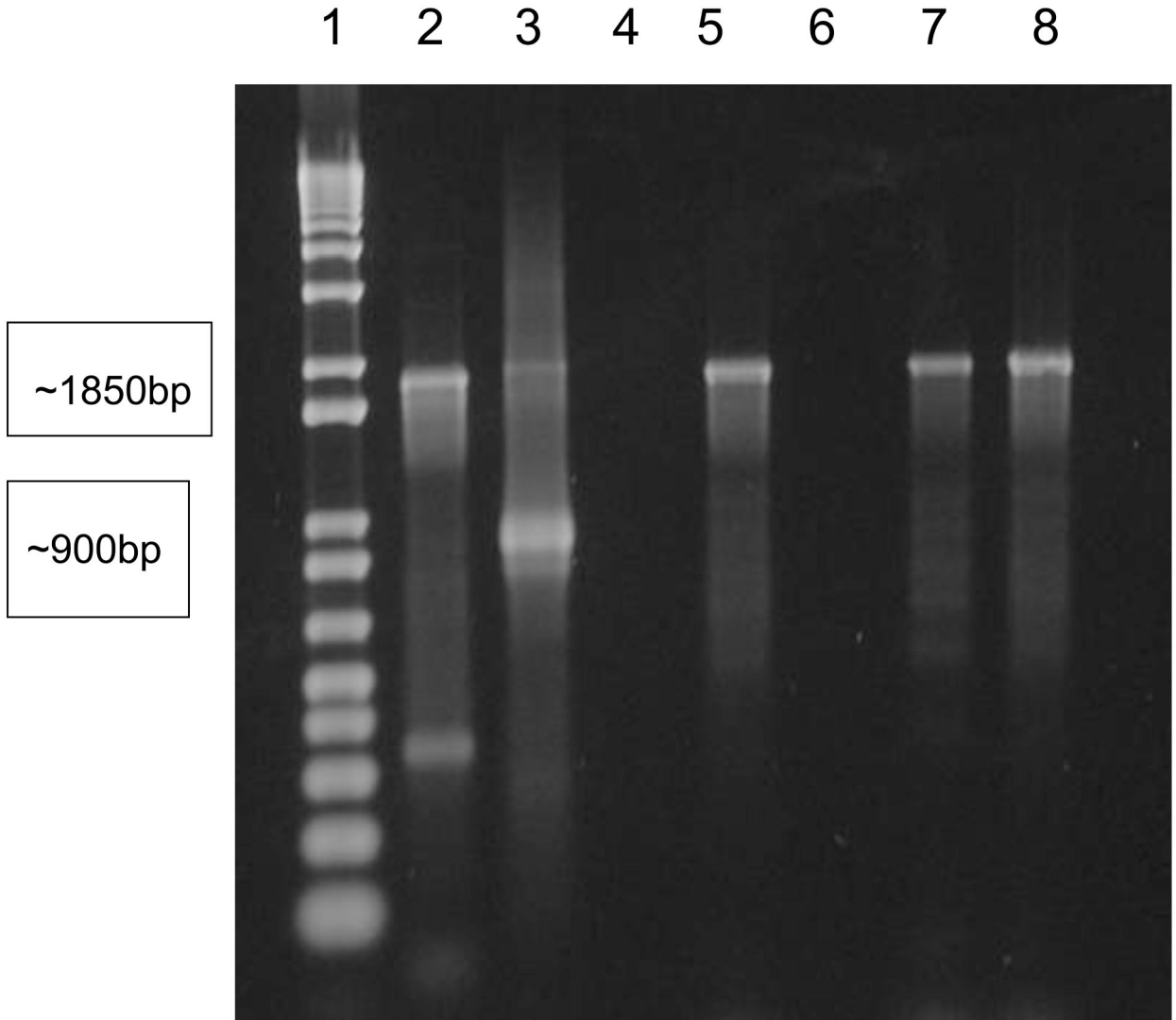
Predicted *omp1* (major outer membrane protein) amino acid sequence deduced from DNA sequences from synovial tissues of 4 representative arthritis patients (#11, 13, 15, 18) compared to the prototype C serovar sequence (C). Nonconserved amino acids are indicated by bold underlined capital letters. Patient samples are from Tampa (patient 18) and from Philadelphia (patients 11, 15, 13).



Patient 2	<u>CAGCACCTTTATCACACGGAGAAAAATCTCC</u> ---TTTTGAAGATCTTTTAGCTGTAGGAT	61
Standard	<u>CAGCACCTTTATCACACGGAGAAAAATCTCC</u> ATTTTTGAAGATCTTTTAGCTGTAGGAT	2324
Patient 2	<u>TGGATCCTATTTTGCTTATTTCTGCAGGGACAACGCCGGAGCGGATGTCTTTAATAACAAG</u>	121
Standard	<u>TGGATCCTATTTTGCTTATTTCTGCAGGGACAACGCCGGAGCGGATGTCTTTAATAACAAG</u>	2384
Patient 2	<i>AATACGCAAGAGGCTTTCTGTATTATATCCCATATCAAGCTACGAGAGATTCTGAAGTAG</i>	181
Standard	<i>AATACGCAAGAGGCTTTCTGTATTATATCCCATATCAAGCTACGAGAGATTCTGAAGTAG</i>	2444
Patient 2	<i>GTATCAAAGAAGAATTTGAAAAGTCAGAGAACATTTTGATCTTCCAATTGTAGATAGAA</i>	241
Standard	<i>GTATCAAAGAAGAATTTGAAAAGTCAGAGAACATTTTGATCTTCCAATTGTAGATAGAA</i>	2504
Patient 2	<i>GAGATATTTGTGATAAAAAAGAAGCTGCACATGTGCTGAATTATTCAGATGGTTTCATTG</i>	301
Standard	<i>GAGATATTTGTGATAAAAAAGAAGCTGCACATGTGCTGAATTATTCAGATGGTTTCATTG</i>	2564
Patient 2	TGAAA 306	
Standard	TGAAA 2569	

**Figure 3.**

The DNA sequence of one *trpA* clone from patient 2 displaying recombined elements containing in part the ocular type sequence (underlined) and in part the Group II genital type sequence (italics). PCR amplification, cloning, and sequence determination were done as described in Patients and Methods.



**Figure 4.**

Representative PCR amplifications to assess the deletion in the plasticity zone on the *C. trachomatis* chromosome, in the region of the cytotoxin locus. PCR amplification, and display on standard 1% agarose gels, were as described in Patients and Methods. Lanes are: 1, 1 kbp size stds; 2, positive control (A serovar DNA as template); 3, positive control (K serovar DNA as template); 4, negative control (water as template); 5, 7, 8, DNA from patients 1, 11, 29; 6, DNA from an irrelevant (PCR-negative) patient. Ocular serovars produce a PCR product of about 1850 bp, while Group I genital serovars produce a PCR product of about 900 bp. See [22].

Table 1

Characteristics of patients from whom samples were procured

Patient	Age/sex	Working Diagnosis	Disease Duration (mo)	Serovar	tpA	Cytotoxin Region
1	27/F	ReA <sup>a</sup>	1	C	nd <sup>b</sup>	ocular
2	51/M	ReA	na	C	ocular+genital <sup>†</sup>	-----c
3	?/F	UO <sup>d</sup>	6	C	nd	-----
4	44/M	ReA	11	C	ocular	ocular
5	47/M	UO	11	C	ocular	ocular
6	?/F	UO	Na	C	nd	-----
7	38/M	UO	15	A	ocular	-----
8	58/M	RA <sup>e</sup>	84	C	nd	ocular
9	33/F	UO	6	C	ocular	-----
10	25/F	UO	na	C	nd	-----
11	32/M	RA	7	C	ocular	ocular
12	39/M	RA	12	A	ocular	-----
13	32/M	UO	0.5	C	ocular	ocular
14	?/M	UO	96	C	nd	ocular
15	44/M	ReA	2	C	ocular <sup>‡</sup>	-----
16	37/M	ReA	na	C	ocular	ocular
17	26/M	ReA	na	C	nd	-----
18	58/F	UO	75	C	ocular	-----
19	63/M	ReA	25	C	ocular	-----
20	47/F	ReA	16	C	nd	-----
21	58/M	ReA	5	C	ocular	-----
22	61/F	RA	7	C	ocular <sup>§</sup>	-----
23	54/F	ReA	na	C	ocular <sup>§</sup>	-----
24	49/F	ReA	na	C	ocular	-----
25	45/F	ReA	na	C	ocular	-----

Patient	Age/sex	Working Diagnosis	Disease Duration (mo)	Serovar	<i>trpA</i>	Cytotoxin Region
26	60/M	OA <sup>d</sup>	na	C	nd	-----
27	68/M	RA	4	C	ocular	-----
28	44/M	ReA	2	C	ocular	-----
29	55/F	RA	na	C	nd	ocular
30	53/F	ReA	na	C	ocular <sup>‡</sup>	ocular
31	54/F	UO	na	C	nd	-----
32	57/F	OA	na	C	ocular	-----
33	55/F	RA	na	C	ocular	-----
34	49/F	RA	na	C	ocular	-----
35	82/F	RA	na	C	ocular	-----
36	58/F	ReA	na	B	nd	-----

<sup>d</sup>ReA, reactive arthritis

<sup>b</sup>not done due to lack of material

<sup>c</sup>not done due to lack of material, or no PCR product produced

<sup>d</sup>UO, undifferentiated oligoarthritis

<sup>e</sup>RA, rheumatoid arthritis

<sup>f</sup>OA, osteoarthritis

<sup>‡</sup>ocular sequences linked to genital sequences; see Figure 3

<sup>‡</sup>most *trpA* DNA sequences of the ocular type but some proportion Group I genital type [22]

<sup>¶</sup>most *trpA* DNA sequences of the ocular type but some proportion Group II genital type [22]