

LIGASE CHAIN REACTION IN DETECTION OF *CHLAMYDIA* DNA IN SYNOVIAL FLUID CELLS

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SUMMARY

Synovial fluid cells from 12 patients with reactive arthritis (ReA) triggered by *Chlamydia trachomatis* were studied for the presence of *Chlamydia* DNA using the ligase chain reaction (LCR) LCx® (Abbott) and the polymerase chain reaction (PCR) Amplicor (Roche). In addition, peripheral blood leucocytes from 11 of these patients were analysed by LCR. As controls, seven patients with newly diagnosed rheumatoid arthritis (RA) were included. *Chlamydia trachomatis* DNA was detectable by LCR in samples of synovial fluid cells from 4/12 patients with *C. trachomatis*-triggered ReA, and in none by PCR. *Chlamydia trachomatis* DNA was not detectable in the synovial fluid cells of the seven RA patients by either method, neither was *C. trachomatis* DNA detectable in the peripheral blood leucocytes of the ReA patients (0/11) or controls (0/6) by LCR. The LCR technique may be useful in the demonstration of *Chlamydia* DNA in synovial fluid cells.

KEY WORDS: LCR, PCR, Reactive arthritis, *Chlamydia trachomatis*.

In reactive arthritis (ReA) triggered by *Chlamydia trachomatis*, microbial components have been identified in the synovial membrane and synovial fluid cells. Two early papers even report the culture of *Chlamydia* from the joint of five patients [1, 2]. There are several reports on the presence of *C. trachomatis*-specific nucleic acids [3–7] at the site of inflammation. Negative results have also been published, concerning *Chlamydia* isolation [8, 9], as well as the presence of DNA [9–11]. Three recent studies [5–7] reporting the occurrence of *C. trachomatis* DNA in the joint tissue or synovial fluid used a technique based on the nested polymerase chain reaction (PCR). However, in two of these studies [5, 6], *C. trachomatis* DNA was also found in two control patients; one had osteoarthritis secondary to patellar fracture [6] and one was initially diagnosed as rheumatoid arthritis (RA) [5]. Additionally, Bas *et al.* [6] observed *Chlamydia* DNA in synovial fluid in 41% (11/27) of patients with undifferentiated seronegative oligoarthritis; the patients had no previous evidence of *Chlamydia* infection. These observations lead one to question the value of molecular amplification, particularly that of nested PCR, in the diagnosis of *Chlamydia*-triggered ReA. This is unfortunate, since the usefulness of one-step PCR in the diagnosis of Lyme arthritis has been demonstrated so elegantly [12].

In the present work, we have used a commercial application of the ligase chain reaction (LCR) [13], a novel method with similarities to the PCR [14], to study

synovial fluid cells from 12 patients with ReA triggered by *C. trachomatis*. The PCR methodology was also utilized.

PATIENTS AND METHODS

The study samples (Table I) were collected during the years 1988–1995. The samples of synovial fluid were drawn from a knee joint of all patients. The diagnosis of the 12 patients with *C. trachomatis*-triggered ReA [10 male, two female; mean age 27 yr (range 16–48 yr); duration of disease 2 months (10 days–11 months)] was based on clinical examination by a rheumatologist and detection of *Chlamydia* antigen or isolation of *C. trachomatis* from the urethral or cervical swab (patients 1–9). In three patients, the diagnosis was based on suspicion of sexually acquired ReA and/or on the presence of circulating *Chlamydia*-specific IgG antibodies (patients 10–12). The patients did not have circulating IgM, IgA or IgG class antibodies against *Yersinia*, *Salmonella* or *Campylobacter*.

As controls, seven patients with newly diagnosed RA [15] were included (Table I). Five of these patients were male and two female; mean age 54 yr (29–73 yr); duration of disease 8 months (5–12 months) at the time of sample collection.

Mononuclear cells of blood and synovial fluid were separated using Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation. In three patients (patients 1, 4 and 8), the synovial fluid cells were pelleted by centrifugation without the Ficoll treatment. From two patients (patients 5 and 11), the polymorphonuclear cell fraction of synovial fluid was studied. From all blood samples as well as from synovial fluid of the other patients and controls (patients 13–19), the mononuclear cell fraction was used. The DNA extraction was performed by

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TABLE I
Clinical characteristics of the patients*

Patient	Age/sex	HLA-B27	Time from onset of arthritis	Urethral or endocervical <i>Chlamydia</i> antigen	LCR result		PCR result
					SFC	PBMC	SFC
<i>Chlamydia</i> -triggered reactive arthritis							
1	25/F	—	1 month	+	—	—	—
2	21/M	ND	3 months	+	+	—	—
3	48/M	ND	3 weeks	+	—	—	—
4	20/M	—	10 days	+ †	+	—	—
5	16/M	ND	1 month	+	—	—	—
6	21/M	+	6 weeks	+	—	—	—
7	21/M	—	3 weeks	+	—	—	—
8	31/M	—	5 weeks	+	—	ND	—
9	26/F	+	3 weeks	+	—	—	—
10	29/M	ND	2 months	ND	+	—	—
11	28/M	—	2 weeks	ND	—	—	—
12	43/M	ND	11 months	—	+	—	—
Rheumatoid arthritis‡							
13	59/M	ND	10 months	ND	—	—	—
14	41/M	ND	5 months	ND	—	—	—
15	61/F	ND	6 months	ND	—	ND	—
16	56/M	ND	5 months	ND	—	—	—
17	61/M	ND	12 months	ND	—	—	—
18	73/F	ND	12 months	ND	—	—	—
19	29/M	ND	5 months	ND	—	—	—

*LCR, ligase chain reaction; PCR, polymerase chain reaction; SFC, synovial fluid cells; PBMC, peripheral blood mononuclear cells; ND, not done.

†Positive by *Chlamydia* culture.

‡All rheumatoid arthritis patients fulfilled the I–IV and VI criteria for RA [15]; 5/7 fulfilled criterion VII; none had rheumatoid nodules.

phenol–chloroform–isoamyl alcohol extraction [16]. The DNA samples were divided into aliquots, coded and analysed blindly by *C. trachomatis*-specific LCR and PCR assays.

The LCx[®] (Abbott, Abbott Park, IL, USA) is based on the LCR methodology and Amplicor (Roche, Branchburg, NJ, USA) on the PCR. Both assays target the cryptic plasmid DNA of *C. trachomatis*. The assays were performed as described by the manufacturer, with the exception that handling of the samples was performed as described above. As a methodological control, a 268 bp segment of the human β -globin encoding genome was amplified to verify the presence of human DNA in the study samples and to exclude inhibitory factors [16].

RESULTS

Chlamydia trachomatis DNA was detectable by LCR in samples of synovial fluid cells from 4/12 patients with *C. trachomatis*-triggered ReA, and in none by the PCR method used. *Chlamydia trachomatis* DNA was not detectable in the synovial fluid cells of the seven RA patients by either method. *Chlamydia trachomatis* DNA was detectable neither in the peripheral blood leucocytes of the ReA patients (0/11) nor in those of controls (0/6) by LCR (Table I). The human β -globin gene was amplified from all samples, indicating that DNA isolation had been properly carried out and that significant inhibitory agents were not present.

DISCUSSION

To our knowledge, this is the first report where bacterial DNA has been demonstrated in synovial fluid

samples by the LCR technique. *Chlamydia trachomatis* DNA was detectable in the cells of synovial fluid in 4/12 patients initially diagnosed as having *C. trachomatis*-triggered ReA. No *C. trachomatis* DNA was detected by LCR in the synovial samples of the seven control patients or in the peripheral blood cells of either patient group. In our hands, the PCR technique used failed to detect any *C. trachomatis* DNA from the samples studied.

The amount of *C. trachomatis* DNA in the fluid of the inflamed joint is probably very low. The sensitivity of the one-step PCR seems not to be adequate for the detection of *C. trachomatis* in the synovial samples [10, 11], as also demonstrated by our study. In the three reports where *C. trachomatis* DNA was observed in the synovial samples, the methodology of nested PCR was applied (5–7). One should note, however, that use of the nested PCR is laborious and may increase the risk of false-positive results.

In a recent report, the LCR and PCR were compared in the detection of *C. trachomatis* in urogenital specimens [17]. The *C. trachomatis* LCR was considered a good alternative test in the diagnosis of chlamydial infections. We conclude that the LCR technique should be evaluated further in the demonstration of *Chlamydia* and other infectious agents in synovial fluid cells.

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