SHORT REPORT

Chlamydophila pneumoniae infection of the central nervous system in patients with multiple sclerosis

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J Neurol Neurosurg Psychiatry 2004;**75**:152–154

Background: *Chlamydophila pneumoniae* has been postulated as an aetiological agent in the pathophysiology of multiple sclerosis. Previous studies show conflicting results.
Objective: To investigate patients with multiple sclerosis and other neurological diseases for evidence of past or present infection with *C pneumoniae*.
Methods: 19 patients with multiple sclerosis and 29 with other neurological diseases were studied. Evidence was sought for past or present infection with *C pneumoniae* using polymerase chain reaction (PCR) and cell culture of cerebrospinal fluid (CSF), and enzyme linked immunosorbent assay and microimmunofluorescence of serum.
Results: *C pneumoniae* was grown from the CSF of one patient with multiple sclerosis. PCR was negative in all cases. Anti-chlamydial antibodies were detected in the same proportion in each group.
Conclusions: This study does not support the theory of an association between *C pneumoniae* and multiple sclerosis.

Chlamydophila pneumoniae is an obligate intracellular pathogen primarily associated with respiratory disease. It is capable of causing persistent or latent infections. A possible association between *C pneumoniae* and multiple sclerosis has been investigated,[1–21] with conflicting results. We investigated a group of 19 patients with multiple sclerosis and 29 control patients with other neurological diseases. We looked for evidence of past or present infection with *C pneumoniae*, using polymerase chain reaction (PCR) and cell culture in cerebrospinal fluid (CSF), and enzyme linked immunosorbent assay (ELISA) and microimmunofluorescence (MIF) in serum.

METHODS

The study group consisted of 48 consecutive patients undergoing routine lumbar punctures for diagnostic purposes. Nineteen had a clinical diagnosis of multiple sclerosis (mean age 50.7 years, 10/19 (52.6%) male); 29 had other neurological diseases (mean age 39.8 years, 17/29 (58.6%) male). Half the control group had inflammatory diseases such as encephalitis or neurosarcoidosis, and half had non-inflammatory diseases such as dementia or epilepsy. Full ethical approval was obtained before the study.

CSF was collected directly into processing tubes and cultured on the day of collection onto HL[22] and HEp2[23] cell lines, using a modification of Sriram's method.[1] Cell monolayers were examined on day 7 and day 14 for chlamydial inclusions using the IMAGEN direct immunofluorescent kit (Dako). The positive isolate was identified by analysis of the *omp2* gene.[24]

For PCR, DNA was extracted using a modification of the method of Sriram,[1] with the addition of an internal control (50 elementary bodies of *C trachomatis* added per sample). Analysis was done using a sensitive and specific PCR-ELONA (enzyme linked oligonucleotide assay).[24]

Serum from all patients was assayed by ELISA for IgM and IgG antibodies to the family *Chlamydiaceae* (MEDAC Diagnostika; interpretation according to manufacturer's instructions). Samples positive by ELISA were further tested using an in-house microimmunofluorescence (MIF) assay, a sensitive and specific assay for anti-chlamydial antibodies that can distinguish between serotypes. We assigned high cut off values in order to exclude non-specific results (cut off titre, 256 for IgG; 64 for IgM and IgA).

A review of published reports was carried out following a Medline search, using the search terms ''*C pneumoniae*'' and ''multiple sclerosis.''

RESULTS

Culture

One CSF sample of the 48 tested was culture positive for *C pneumoniae*. The CSF came from a patient with multiple sclerosis (see case report below). Direct immunofluorescent staining showed inclusion bodies in HL and HEp2 monolayers at seven days. The number of inclusions seen at the first passage was very scanty, but improved after several further passages. The positive culture result was confirmed by repeating the culture using an untouched aliquot of CSF from the same patient, which had been taken at the same time as the original sample and frozen at −70℃. *C pneumoniae* was again grown from the CSF. PCR-RFLP and DNA sequencing of the partial *omp2* gene product confirmed the isolate as *C pneumoniae*.

PCR

C pneumoniae DNA was not detected by PCR-ELONA in any patient sample. All external negative and positive extraction PCR and ELONA controls were valid. The internal control was not detected in four samples, including the culture positive sample.

Serology

Nine of the 19 patients with multiple sclerosis (47.4%) were antibody positive by ELISA. Four of these nine (44%) were positive by MIF for antibodies to *C pneumoniae*. Fifteen of 29 control patients (51.7%) were antibody positive by ELISA. Six of these 15 (40%) were positive by MIF for antibodies to

Abbreviations: MIF, microimmunofluorescence; ELISA, enzyme-linked immunosorbent assay; ELONA, enzyme-linked oligonucleotide assay; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

Table 1 Review of published reports: summary of results of cerebrospinal fluid (CSF) polymerase chain reaction (PCR), culture, and serology

Paper	CSF PCR, MS	CSF PCR, control	CSF PCR, p value	CSF culture, MS	CSF culture, control	CSF culture, p value	CSF serology, MS	CSF serology, control	CSF serology, p value	Serum serology, MS	Serum serology, control	Serum serology, p value
Sriram *et al*, 1999[1]	36/37 (97%)	5/27 (19%)	<0.001	24/37 (64%)	3/27 (11%)	<0.001	32/37 (86%)	4/26 (15%)	<0.001	–	–	–
Boman *et al*, 2000[3]	0/48 (0%)	0/51 (0%)	NS	0/48 (0%)	0/51 (0%)	NS	–	–	–	26/38 (68%)	33/40 (82%)	–
Dong-Si *et al*, 2000[4]	12/18 (66%)	4/19 (21%)	0.014	–	–	–	–	–	–	–	–	–
Layh-Schmitt *et al*, 2000[5]	7/30 (23%)	0/56 (0%)	<0.001	2/8 (25%)	–	–	2/30 (7%)	2/56 (4%)	NS	*	*	*
Li *et al*, 2000[6]	9/17 (52%)	13/15 (86%)	NS	–	–	–	–	–	–	–	–	–
Morre *et al*, 2000[7]	0/27 (0%)	0/36 (0%)	NS	–	–	–	–	–	–	–	–	–
Parratt *et al*, 2000[8]	–	–	–	–	–	–	3/20 (15%)	0/5 (0%)	NS	–	–	–
Poland & Rice, 2000[9]	0/175 (0%)	0/40 (0%)	NS	–	–	–	–	–	–	–	–	–
Pucci *et al*, 2000[10]	0/29 (0%)	0/7 (0%)	NS	–	–	–	–	–	–	–	–	–
Treib *et al*, 2000[11]	2/8 (25%)	–	–	–	–	–	8/22 (36%)	–	–	–	–	–
Derfuss *et al*, 2001[12]	0/13 (0%)	0/10 (0%)	NS	–	–	–	11/46 (24%)	3/61 (5%)	0.027	23/45 (51%)	32/60 (53%)	NS
Gieffers *et al*, 2001[13]	12/58 (21%)	20/47 OND (43%) or 0/67 healthy controls (0%)	–	0/3 (0%)	1/11 (9%)	NS	–	–	–	–	–	–
Krametter *et al*, 2001[14]	–	–	–	–	–	–	16/52 (31%)	1/43 (2%)	–	*	*	*
Numazaki & Chibar, 2001[15]	0/5 (0%)	–	–	–	–	–	–	–	–	–	–	–
Pincherle *et al*, 2001[16]	23/107 (21%)	2/77 (3%)	0.0002	–	–	–	–	–	–	*	*	*
Saiz *et al*, 2001[17]	0/19 (0%)	0/20 (0%)	NS	–	–	–	3/19 (16%)	0/20 (0%)	–	10/19 (53%)	15/20 (75%)	–
Sotgiu *et al*, 2001[18]	3/32 (9%)	0/30 (0%)	–	–	–	–	3/32 (9%)	4/30 (13%)	–	–	–	–
Hao Q *et al*, 2002[19]	9/28 (32%)	2/15 (13%)	NS	1/28 (4%)	1/15 (7%)	–	13/66 (20%)	1/25 (4%)	0.064	43/66 (65%)	16/25 (64%)	–
Kaufman *et al*, 2002: VUMC[20]†	22/30 (73%)	5/22 (23%)	<0.001	–	–	–	–	–	–	–	–	–
Kaufman *et al*, 2002: JHU[20]†	0/30 (0%)	0/22 (0%)	NS	–	–	–	–	–	–	–	–	–
Kaufman *et al*, 2002: UU[20]†	0/30 (0%)	0/22 (0%)	NS	–	–	–	–	–	–	–	–	–
Kaufman *et al*, 2002: CDC[20]†	0/14 (0%)	0/5 (0%)	NS	–	–	–	–	–	–	–	–	–
Munger *et al*, 2003[21]	–	–	–	–	–	–	–	–	–	106/141 (75%)	184/282 (65%)	0.03
Furrows *et al*, 2003‡	0/19 (0%)	0/19 (0%)	NS	1/19 (5.3%)	0/29 (0%)	NS	–	–	–	4/19 (21%)	6/29 (21%)	NS

*Serum serology was done but results not detailed in paper; results described as ''not different'' or ''unrevealing''.
†Samples tested at four different centres.
‡This study.
MS, multiple sclerosis.

C pneumoniae. Antibodies to *C pneumoniae* were therefore detected in the same proportion in each group: four of 19 patients with multiple sclerosis (21%) and six of 29 control patients (21%).

Review of published reports

Results of the literature review are given in table 1.

CASE REPORT

The patient whose CSF was culture positive for *C pneumoniae* was in the multiple sclerosis group of the study. He presented in October 1998, aged 20, with gradual onset of progressive ataxia. In March 1999 he developed weakness of the left arm and numbness of the face and trunk. In August 1999 he developed loss of sensation in the right leg. Magnetic resonance imaging of the head was consistent with multiple sclerosis. Subsequently, isoelectric focusing with immunofixation of the CSF showed the presence of intrathecally synthesised oligoclonal IgG bands. A diagnosis of clinically definitive relapsing-remitting multiple sclerosis was made, supported by radiological findings.

The patient was recruited into this study in December 1999. *C pneumoniae* was grown from his CSF. Anti-chlamydial IgG antibodies were detected by ELISA at a titre of 200. MIF confirmed the presence of IgG antibody to *C pneumoniae*. IgM was not detected by ELISA or MIF, indicating that the

infection was not acute. IgA was not detected by MIF. The serological findings suggest a previous (not acute) infection with *C pneumoniae*.

DISCUSSION

Multiple sclerosis is considered to be the end point of an autoimmune process triggered by an environmental factor in susceptible individuals. Numerous agents have been suggested as the environmental trigger. Sriram's original study in 1999,[1] which appeared to show a convincing link between *C pneumoniae* infection and multiple sclerosis, met with great interest. Subsequent studies by other institutions failed to produce convincing evidence of *C pneumoniae* infection in patients with multiple sclerosis. In order to investigate the association shown by Sriram *et al*, we chose to use similar methodology, concentrating on CSF culture, CSF PCR, and serology.

The most striking result in this study is the culture of *C pneumoniae* from the CSF of a patient with multiple sclerosis. This is the first time it has been cultured from CSF in the United Kingdom, and is only the third clinical isolate from any site in the United Kingdom. Serology confirmed that the culture positive patient had had previous *C pneumoniae* infection. PCR was negative from this sample and all others. The PCR incorporated an internal control, which proved that 90% of individual assays were able to detect at least 125 genomes/ml CSF. As the routine extraction, external PCR, and ELONA controls were valid, a negative result has been given on all samples. However, samples with negative internal controls could be reported as ''no result'' as the level of sensitivity is unknown. The internal control was not detected in the culture positive sample, explaining the discrepancy between the positive culture and negative PCR result.

Our literature review showed that results of different studies were highly inconsistent. Following the original study by Sriram *et al*,[1] most but not all studies failed to detect *C pneumoniae* in patients with multiple sclerosis. A study by Kaufman *et al*,[20] in which the same samples were tested at four different centres, showed great variation in results between centres. Therefore these differences cannot be attributed solely to differences in study populations but may also reflect differences in the collection and storage of samples, the DNA extraction method, or the culture/PCR techniques.

Conclusions

While we were able to culture *C pneumoniae* from the CSF in one patient of 19 with multiple sclerosis, serology did not show any difference in exposure between the subject groups, and PCR was negative in all cases. While the isolate is a unique culture result in the United Kingdom, our data do not support the hypothesis that *C pneumoniae* is implicated in the pathogenesis of multiple sclerosis. However, the recent nurses' health study[21] suggests there may be a positive association between *C pneumoniae* infection and multiple sclerosis, particularly progressive multiple sclerosis. There is a need for well designed clinical and epidemiological studies to produce more direct evidence for a role of *C pneumoniae* infection in multiple sclerosis.

ACKNOWLEDGEMENTS

This project was supported by a generous grant from the MS Society of Great Britain and Northern Ireland. Our thanks to Hussan Salman for technical advice.

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Competing interests: none declared

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Received 28 November 2002
In revised form 7 April 2003
Accepted 21 May 2003

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