Chlamydia pneumoniae in children with MS: Frequency and quantity of intrathecal antibodies

K. Rostasy, H. Reiber, D. Pohl, P. Lange, A. Ohlenbusch, H. Eiffert, M. Maass and F. Hanefeld

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neuroimaging studies should be considered when patients present with a new onset of BDD.

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Chlamydia pneumoniae in children with MS

Frequency and quantity of intrathecal antibodies

K. Rostasy, MD; H. Reiber, PhD; D. Pohl, MD; P. Lange, BSc; A. Ohlenbusch, PhD; H. Eiffert, MD; M. Maass, MD; and F. Hanefeld, MD

Abstract—The authors investigated the frequency and quantity of intrathecal antibody synthesis against *Chlamydia pneumoniae* and the presence of *C pneumoniae* antigen in 25 children with MS. *C pneumoniae* genome was present in two children. In seven children an intrathecal synthesis of *C pneumoniae* antibodies was detected, representing only a small part of the total intrathecal immunoglobulin G, suggesting that this intrathecal synthesis is part of a polyspecific, oligoclonal immune response.

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The intrathecal synthesis of immunoglobulins (Ig) is a hallmark in MS. It is characterized by the presence of polyspecific, intrathecal production of antibodies against a wide range of antigens, including *Chlamydia pneumoniae*, recently prompting the question whether this pathogen might be involved in the events leading to MS.[1-4] To further validate a possible link, we analyzed the CSF and serum of 25 children with MS for the presence of *C pneumoniae* genome and the frequency and quantity of the intrathecal *C pneumoniae* IgG antibodies synthesis.

Materials and methods. Patients were admitted to the Pediatric and Adult Neurology Department, University of Göttingen, Germany (1993 to 2001). CSF/serum samples were collected for routine analysis. Group 1 included 25 children with MS according to the Poser criteria (table 1).[5] Group 2 included 10 patients with various other inflammatory neurologic disorders (OIND; table 2). Group 3 included 18 patients with other noninflammatory neurologic disorders (OND; see the supplementary table at www.neurology.org).

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C pneumoniae *PCR.* In the Department of Microbiology, University of Göttingen, 44 CSF samples were analyzed by PCR targeting the 16SrRNA and rpoB genes. In the first PCR, the following primers were used: CpnA(for)5'TGACAA CTGTAGAAATAC AGC3', CpnB(rev) 5'CGCCTCTCTCCT ATAAAT3'. In the second PCR, the following primer set was used, yielding a fragment of 420 base pairs (bp): CpnC(for)5'CAA GGACAGATACACAGGTGC3', CpnF(rev)5'GGTTGAGTCA ACGACTTAAGG3'. The rpoB gene was amplified using a seminested PCR technique, yielding a fragment of 350 bp. The subsequent primer sets were used for the first and second round: HL-1(for) 5'GTTGTT CATGAAGGCCTACT3', HR-1(rev)5'TGCATAACCTAC GGTGTCTT3'; HA-2(rev) 5'CTCCGTTAGAGAGATAT GGC3'.

In the Institute of Medical Microbiology, University of Lübeck, 28 of 44 samples were analyzed for *C pneumoniae* by nested PCR based on a previously published protocol.[6]

Detection of C pneumoniae *IgG specific antibody synthesis.* The Antibody Index (AI) is the ratio between the specific *C pneumoniae-*

From the Department of Pediatric Neurology (Drs. Rostasy, Pohl, Ohlenbusch, and Hanefeld), Neurochemistry Laboratory (Drs. Reiber and Lange), and Department of Bacteriology (Dr. Eiffert), University of Göttingen; and Institute of Medical Microbiology and Hygiene (Dr. Maass), University of Lübeck, Germany.

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Address correspondence and reprint requests to Kevin Rostasy, Pediatric Neurology, Georg-August-University of Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen, Germany; e-mail: Krostasy@excite.com

Table 1 *Detection of intrathecal* Chlamydia pneumoniae *genome and antibodies in 25 children and adolescents with MS, all with oligoclonal IgG in the CSF*

Patient	Age, y/sex	Cells/μL	IF_{IgG}, %	IF_{IgM}, %	AI/M*	AI/R	AI/VZV	AI/Chl	$PCR/CSF_{Gö/Lü}$†	Chl/serology
1	14/F	15	0	0	1.2	0.8	1.3	3.1	−/ND	IgG, IgA
2	14/F	12	45	0	—‡	1.2	2.0	—	−/ND	—
3	18/M	0	70	0	4.9	10.1	0.8	1.6	−/−	IgG
4§	13/F	8	0	0	—	0.9	—	1.3	−/ND	IgG
5	16/F	28	0	34	—	0.7	0.8	0.7	−/ND	IgG, IgA
6	15/F	4	13	63	0.8	0.9	0.8	—	−/−	—
7	17/F	1	22	45	—	3.2	1.0	—	−/−	—
8	12/F	ND	35	0	1.9	0.6	1.5	—	−/ND	—
9	13/F	1	0	0	1.4	6.8	15.5	—	−/ND	—
10§	16/F	6	26	0	2.5	4.1	0.8	2.1	−/−	IgG
11	18/F	ND	0	0	0.9	—	0.8	—	+/+	IgG
12	17/F	ND	0	0	0.8	1	—	—	−/−	—
13	14/F	50	68	34	3.4	9	8.0	—	−/ND	—
14	9/M	4	0	85	42.0	7	—	1.1	−/ND	—
15	17/F	9	54	75	3.3	—	1.4	2.0	−/−	IgG, IgM
16	15/F	0	0	0	1.2	1.6	1.7	1.0	−/−	IgG
17	14/F	10	51	0	—	1.0	5.4	1.6	−/ND	IgG
18	12/F	11	72	63	7.2	1.1	6.8	1.5	−/−	IgG
19	18/F	2	40	0	1.0	3.0	2.1	—	+/+	IgM
20	16/F	9	60	0	5.9	1.4	5.6	2.6	−/−	IgM
21	14/F	4	0	0	4.4	0.7	1.4	0.7	−/ND	IgG, IgM, IgA
22	18/F	13	11	40	19.6	2.1	6.5	0.7	−/ND	IgG
23	18/F	6	0	0	—	15.9	—	1.0	−/ND	IgG
24	14/F	19	30	0	10.6	1.1	1	1.3	−/−	IgG
25¶	13/F	7	0	0	3.5	0.6	—	1.0	−/ND	IgG, IgM, IgA

* AI = Antibody Index for M (measles), R (rubella), VZV (varicella zoster virus), and Chl (*C pneumoniae*).
† PCR performed in two different laboratories: Gö (Göttingen) and Lü (Lübeck).
‡ − = Value below the level of the method.
§ Primary progressive MS.
¶ IF_{IgA} = 57%; in all other samples IgA was not detected.

Ig = immunoglobulin; IF = Intrathecal fraction; ND = not determined.

CSF/serum quotient, Q_{spec}, calculated as $Q_{spec} = AU_{CSF}/AU_{Ser}$, and the total CSF/serum immunoglobulin quotient (Q_{IgG}): $AI = Q_{spec}/Q_{IgG}$ (AU = arbitrary concentration units). The method-dependent, normal reference range of AI is 0.7 to 1.3 (1.0 ± 2SD) and pathologic AI values are clinically defined as AI > 1.4.[1,2]

The antibody concentration of *C pneumoniae* was detected with an ELISA from Euroimmun (Lübeck, Germany).[1,2] The greatest measurable standard concentration with an absorbance of approximately OD = 2 was defined to be 100 AU. The difference of absorbance (ΔA) was measured with a microtiter plate reader (SLT Labinstruments). ΔA for the negative control was subtracted from each patient sample ΔA and only OD values greater than 0.1 were used for calculation. The normal range of AI values (0.7 to 1.3) was detected with 18 pairs of CSF/serum samples from patients without inflammatory CNS disease. The absolute *C pneumoniae* antibody concentrations in CSF, instead of AU as used for the AI, have been detected by a particular ELISA method (see the supplementary Methods section at www.neurology.org for further details).[7]

Results. Oligoclonal bands in the CSF were detected in all 25 patients with MS. An intrathecally synthesized Ig fraction for IgG, IgM, or IgA was detectable in 17/25 (68%) subjects (see table 1).

In 18/25 (76%) patients with MS, at least one of the three antibody species—measles (12/25), rubella (10/25), or varicella (9/25)—was intrathecally synthesized (AI > 1.4). Specific antibodies against *C pneumoniae* were detected in 7/25 (28%) patients with MS.

In 3/10 patients with OIND, oligoclonal bands were detected (see table 2). Specific antibodies against *C pneumoniae* were not present.

Patients with OND had no oligoclonal bands, no intrathecal Ig synthesis, and no intrathecal synthesis of rubella, measles, varicella, or *C pneumoniae* antibodies (see the supplementary table at www.neurology.org).

In 7 of 25 children with MS and an intrathecal synthesis of *C pneumoniae* antibodies, the amount of *C pneumoniae* antibodies was calculated as % of the intrathecally synthesized total IgG. As shown in table 3, *C pneumoniae* antibodies were a small fraction between 0.01% and 0.8% (median 0.04%) of the total intrathecally synthesized IgG.

Further, we studied the serum and CSF of five adult

Table 2 *Detection of intrathecal* Chlamydia pneumoniae *genome and antibodies in 10 children and adolescents with inflammatory diseases of the CNS other than MS*

Patient	Diagnosis	Age, y/sex	Cells/μL	OB	AI/M*	AI/R	AI/VZV	AI/Chl	PCR/CSF$_{Gö/Lü}$†	Chl/serology
26	ADEM	6/M	2	+	0.9	0.9	—	—	−/ND	—
27	ADEM	16/M	2	−	—	0.9	—	1.4	−/ND	IgG
28	ADEM	14/M	5	−	—	1.1	1.6	—	−/−	—
29	ADEM	15/F	ND	+	—	—	2.7	0.7	−/ND	IgG
30	Optic neuritis	18/F	4	−	—	—	—	—	−/−	IgM
31	Optic neuritis	12/F	1	−	—	—	0.8	0.8	−/−	IgG
32	Optic neuritis	12/M	2	−	—	—	—	—	+/+	IgG, IgM
33	CNS vasculitis	14/F	2	+	—	1.3	1.4	—	−/−	IgG
34	CNS vasculitis	14/F	ND	−	1.1	1.4	1	—	−/ND	—
35	T-cell encephalitis	15/F	6	−	—	0.9	—	—	−/−	—

* AI = Antibody Index for M (measles), R (rubella), VZV (varicella zoster virus), and Chl (*C pneumoniae*).
† PCR performed in two different laboratories: Gö (Göttingen) and Lü (Lübeck).

OB = oligoclonal bands; ADEM = acute disseminating encephalomyelitis; ND = not determined; Ig = immunoglobulin.

patients with MS with a prominent intrathecal synthesis of IgG. In all five patients, the quantity of *C pneumoniae* antibodies was higher compared to the seven children with MS (range 0.24% to 5.1%; median 0.9%).

C pneumoniae genome in CSF was detected in 2 of 25 patients with MS and one patient with OIND (Patients 11 and 19, table 1; Patient 35, table 2). Two of three cases were positive for IgM antibodies against *C pneumoniae* in serum, indicating an acute infection with *C pneumoniae* (Patient 19, table 1; Patient 35, table 2). In patients with OND, the first laboratory reported no positive PCR. The second laboratory detected *C pneumoniae* genome in two patients with OND (Patients 38 and 40; see the supplementary table at www.neurology.org).

Discussion. Our results indicate that the intrathecal presence of *C pneumoniae* antibodies in a subgroup of young patients with MS is part of a polyspecific, oligoclonal immune response rather than the consequence of an acute/persistent infection, based on the following findings.

In most subjects, *C pneumoniae* genome was not present in the CSF and was only detected in two children with MS.

An intrathecal synthesis of *C pneumoniae* antibodies was seen in 7/25 (28%) patients. However, in contrast to the extent of the AI values, the quantity of intrathecal synthesis of the single antibody is more

Table 3 Chlamydia pneumoniae *antibody concentration in serum and CSF, antibody index, and quantification of intrathecal synthesis in seven adolescents and five adult patients with MS*

Patient	Age, y/sex	IF$_{IgG}$, %	AI/M*	AI/R	AI/VZV	AI/Chl	Chl$_{CSF}$, mg/L	Chl$_{Loc}$, mg/L	IgG$_{CSF}$/Chl$^{3}_{Loc,\ \%}$
1	14/F	51	—‡	1.0	5.5	1.6	0.0098	0.0037	0.018
3	14/F	0	1.2	0.8	1.3	3.0	0.047	0.031	0.09
10	16/F	26	2.5	4.1	0.8	2.0	0.003	0.016	0.22
15	12/F	72	7.2	1.1	6.8	1.5	0.19	0.064	0.01
17	17/F	54	3.3	1.6	1.4	2.0	0.57	0.29	0.75
18	16/F	60	5.9	1.4	5.6	2.6	0.013	0.008	0.02
20	18/M	70	4.9	10.1	0.8	1.6	0.073	0.027	0.04
1§	26/F	57	3.9	2.0	3.9	10.9	0.37	0.336	0.9
2	28/F	65	—	20.0	11.2	14.1	0.093	0.086	0.24
3	27/M	46	6.5	6.3	2.2	3.0	0.27	0.18	0.41
4	29/F	37	—	1.9	—	4.3	0.21	0.16	1.44
5	49/M	16	1.0	2.1	1.6	5.3	0.53	0.43	5.1

* AI = Antibody Index for M (measles), R (rubella), VZV (varicella zoster virus), and Chl (*C pneumoniae*).
† Chl$_{Loc}$ = local, intrathecal synthesized *C pneumoniae* IgG antibodies.
‡ − = Value below the level of the method.
§ Patients 1 through 5: adult patients with MS with a prominent intrathecal IgG synthesis and a positive M/R/VZV (MRZ) reaction (AI > 1.4).

IF$_{IgG}$ = Intrathecal fraction of immunoglobulin G (IgG).

discriminatory between acute/chronic diseases with a causative antigen vs a disease without a single detectable antigen (e.g., MS). Only 0.01 to 0.8% of the intrathecally synthesized total IgG in children with MS were *C pneumoniae* antibodies, which is in contrast to diseases such as herpes simplex virus encephalitis.[8]

The comparison of the intrathecal antibody synthesis against *C pneumoniae* in children vs adults also supports the idea of a polyspecific immune response. In adult patients with MS with an elevated intrathecal IgG synthesis the likelihood to detect measles, rubella, varicella, and *C pneumoniae* antibodies in parallel is increased,[1] possibly a consequence of a larger set of B-cell clones for different species, which earlier had migrated into the brain.

Although the biologic significance is not understood, this polyspecific, oligoclonal immune response represents an important tool to support the diagnosis of a chronic inflammatory process.

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A novel form of autophagic vacuolar myopathy with late-onset and multiorgan involvement

D. Kaneda, MD; K. Sugie, MD, PhD; A. Yamamoto, MD, PhD; H. Matsumoto, MD; T. Kato, MD, PhD; I. Nonaka, MD, PhD; and I. Nishino, MD, PhD

Abstract—The authors report a 41-year-old man with a novel form of adult-onset autophagic vacuolar myopathy (AVM) with multiple organ involvement including eyes, heart, liver, lung, kidney, and skeletal muscle. The vacuolar membranes had sarcolemmal features similar to vacuoles in Danon disease, X-linked myopathy with excessive autophagy, and infantile AVM. Lysosome associated membrane protein-2, absent in Danon disease, was present. Defined by distinct clinical features, this disease constitutes the fourth entity in the group of autophagic vacuolar myopathy in which the vacuolar membranes have features of sarcolemma.

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Danon disease,[1] X-linked myopathy with excessive autophagy (XMEA),[2] and infantile autophagic vacuolar myopathy (AVM)[3] share a common pathologic feature: autophagic vacuoles with sarcolemmal features. The vacuolar membranes immunostain with antibodies to various sarcolemmal proteins and have acetylcholinesterase (AChE) and nonspecific esterase (NSE) activities. Although the mechanisms by which autophagic vacuoles develop are not known, this unique pathologic finding distinguishes these three diseases from other myopathies.

Danon disease was originally reported as lysosomal glycogen storage disease with normal acid maltase,[1] but was found to be due to a primary deficiency of lysosome associated membrane protein-2 (LAMP-2), a lysosomal structural membrane protein rather than a glycolytic enzyme; therefore, this disease is not a glycogen storage disease.[4] The disease is clinically characterized by hypertrophic cardiomyopathy, myopathy, and mental retardation.[5]

XMEA is clinically characterized by mild slowly progressive proximal dominant myopathy.[2] In this

From the Department of Neurology (Drs. Kaneda and Kato), Osaka Red Cross Hospital; and Department of Neuromuscular Research (Drs. Sugie, Yamamoto, Matsumoto, and Nishino), National Institute of Neuroscience, and National Center Hospital for Mental, Nervous, and Muscular Disorders (Dr. Nonaka), National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan.

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Address correspondence and reprint requests to Dr. Ichizo Nishino, Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo, 187-8502 Japan; e-mail: nishino@ncnp.go.jp

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