Beteiligung des zentralen Nervensystems beim Lupus erythematodes: Eine diagnostische Herausforderung*

Involvement of the Central Nervous System in Lupus Erythematosus: A Diagnostic Challenge*

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Schlüsselwörter: Blut-Hirn-Schranke; IgG/Liquor cerebrospinalis; IgM/Liquor cerebrospinalis; Lupus erythematodes, Systemischer/Liquor cerebrospinalis.

Keywords: Blood-Brain Barrier; IgG/cerebrospinal fluid; IgM/cerebrospinal fluid; Lupus Erythematosus, Systemic/cerebrospinal fluid.

Neurologische und/oder psychiatrische Symptome können im Rahmen eines bestehenden systemischen Lupus erythematodes (LE) oder gelegentlich auch als initiale Manifestation isoliert vorkommen. Angesichts der massiven Schwierigkeiten bei der klinischen Diagnosestellung [1, 2] ist ein Labortest zum Nachweis eines neuropsychiatrischen Lupus (NP-LE) äußerst willkommen. Da man neuropathologisch - im Gegensatz zu Niere und Haut - keine Vasculitis und keine durch Komplementbindung geschädigte Zellen nachweisen konnte, gewann die Vorstellung, daß antineuronale Antikörper für die im allgemeinen reversiblen, diffusen Symptome des NP-LE verantwortlich sein könnten, zunehmend an Beliebtheit. Lokale Defekte in der Blut-Hirn-Schranke infolge von Mikroinfarkten, Hämorrhagien und Vasculopathien, sowie eine erhöhte Permeabilität des Plexus choroideus nach Ablagerung von Immunkomplexen würden einen vermehrten Übertritt von antineuronalen Antikörpern aus dem Blut ins ZNS begünstigen, ganz abgesehen von einer möglichen intrathekalen Produktion solcher Antikörper. Leider steht bis heute, trotz jahrzehntelanger Forschung, kein zuverlässiger, allgemein reproduzierbarer Labortest für die Diagnose eines NP-Lupus zur Verfügung [3]. Weder die mit neuronalen Komponenten kreuzreagierenden lymphozytären Antikörper, noch die diversen Modifikationen zum Nachweis antineuronaler Antikörper erlauben eine eindeutige Identifikation eines NP-LE [4].

Überraschenderweise wurde vor kurzem über Antikörper gegen ein 50 kD Protein aus Synaptosomen berichtet, die bei 19/20 Patienten mit NP-Lupus, aber nur bei 8/23 mit LE ohne neurologische Symptomatik nachgewiesen wurden [5]. In Übereinstimmung mit den Autoren fanden wir solche Antikörper ebenfalls fast ausschließlich bei LE-Patienten, die Sensitivität war jedoch mit 37% viel geringer, und zwischen LE mit und ohne neurologische Beteiligung ergab sich kein signifikanter Unterschied. Große Erwartungen wurden zunächst in die Bestimmung von Antikörpern gegen ribosomales P Protein gesetzt, die für Psychosen bei NP-LE spezifisch sein und sogar mit dem zeitlichen Verlauf korrelieren sollten [6]. Obwohl zwei weitere Arbeitsgruppen diese Ergebnisse partiell bestätigen konnten, fanden vier andere Untersucher keinen Zusammenhang [7].

Ähnlich kontrovers wird der Wert der Liquordiagnostik eingeschätzt. *Futrell* et al. [2] und *Bruyn* [4] halten eine Lumbalpunktion lediglich zur Feststellung einer Infektion für notwendig, wobei sie zu Recht darauf aufmerksam machen, daß eine Pleozytose bei diesen häufig immunsupprimierten Patienten dennoch fehlen kann. Andere Arbeiten [1,8,9,10] dagegen messen der Liquoruntersuchung große differentialdiagnostische Bedeutung bei. Aus der Tabelle 1 wird ersichtlich, daß alle 12 Autoren ähnliche Häufigkeiten (17-44%) für eine Störung der Blut-Liquor-Schranke, ausgedrückt als Albumin CSF/Serum Quotienten finden. Dagegen schwankt das Vorkommen einer quantitativ erhöhten intrathekalen IgG-Synthese von 17-75% und die Häufigkeit von mittels Isoelektrofokussierung nachgewiesenen oligoklonalen Banden von 25-82%. Obwohl unterschiedliche Grenzwerte benutzt wurden und nicht in allen Untersuchungen ein Vergleich zwischen NP-LE und LE ohne neuropsychiatrische Manifestation vorgenommen wurde, läßt sich insgesamt doch der Schluß ziehen, daß eine Schrankenstörung und/oder eine lokale IgG-Produktion als Hinweis für eine ZNS-Beteiligung gewertet werden können.

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*Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic III, Marburg an der Lahn, September 29 and 30, 1995
Nicht standardisierte Abkürzungen: CBB, Coomassie Brillant Blue; IEF, isoelektrische Focussierung; LE, Lupus erythematodes; NP-LE, neuropsychiatrischer Lupus erythromatodes.

Wenn auch ein positiver IgM-Index höhere Häufigkeiten als der IgG-Index beim LP-Lupus aufzuweisen scheint, ist gegenüber diesem Parameter doch besondere Vorsicht angebracht. In den eigenen Untersuchungen erwiesen sich von sieben pathologischen IgM-Indices bei der Berechnung mit der hyperbolischen Formel, die eine bessere Korrektur bei Schrankenstörungen gewährleistet, nur zwei als echt. Überdies wird eine IgM-Erhöhung schon durch geringste Mengen an Blut vorgetäuscht. Die von drei Autoren und von uns selbst gemachte Beobachtung, daß beim LE immer wieder eine quantitative Erhöhung der IgG Synthese trotz fehlender oligoklonaler Banden festgestellt wird, könnte als Indiz für eine intracerebrale Exsudation von Serum gewertet werden. Neuropathologisch konnten Mikroinfarkte bei 35-71% und Hämorrhagien bei 21-42% der LE Patienten nachgewiesen werden [11].

Bei der Heterogenität sowohl der klinischen Syptomatik als auch der pathogenetischen Ursachen erscheint die Forderung nach einem universellen Marker für den NP-LE wenig realistisch. Die diagnostischen und therapeutischen Probleme - Antikoagulation, Immunsuppression, Corticosteroide [4] - bei der Beteiligung des Nervensystems beim LE, erfordern eine am Einzelfall orientierte Kombination klinischer Expertise mit den Ergebnissen der Kernspintomographie und einer Batterie labordiagnostischer Methoden. Eine bessere Zuordnung zu den diversen Labormeßgrößen wäre wahrscheinlich zu erreichen, wenn klinischerseits der komplexe Begriff NP-Lupus, der ein ganzes Spektrum sehr unterschiedlicher Manifestationen mit ebenfalls sehr unterschiedlichen pathogenetischen Prinzipien - Vasculopathien, mit Anti-Phospholipid Antikörpern assoziierte Thrombosen, gegen Neuronen reaktive Antikörper, und durch Zytokine gesteigerte Autoimmunität - umfaßt, zugunsten homogenerer Gruppen aufgegeben würde.

Tabelle 1 Literaturübersicht über Liquorveränderungen beim Lupus Erythematosus

Autoren	N	Pleozytose	Blut/CSF Schranke	IgG Synthese	IgM Synthese	CSF Oligoklonale Banden
Zvaifler und Bluestein [12]			Q_{Alb}[a] > 7,4	IgG/Alb > 26%		Methode nicht angegeben
NP-LE	8		2 (25%)	6 (75%)		0/6 (0%)
LE	6		1 (17%)	0 (0%)		
Seibold et al. [13]			Q_{Alb} > 8,5	IgG Index[b] > 0,58		Immunkomplexe in der Agarose-Elektrophorese
NP-LE	34		1/20 (5%)	10/20 (50%)		33 (97%)
LE	12		0/7 (0%)	0/7 (0%)		1 (8%)
Winfield et al. [14]			Q_{Alb} > 9,0	IgG Index > 0,60		Polyacrylamid-IEF, CBB
schwerer NP-LE	6		5 (83%) } (26%)	} 5/19 (26%)		} 9/19 (42%)
leichter NP-LE	13		0 (0%)			
Hirohata et al. [15]			Q_{Alb} > 9,0	IgG Index > 0,76	IgM Index > 0,20	
NP-LE	13		4 (31%)	9 (70%)	13 (100%)	
LE	7		0 (0%)	0 (0%)	0 (0%)	
Ernerudh et al. [16]		> 5 Zellen/µl	Q_{Alb} > 7,4	IgG Index > 0,70	IgM Index > 0,06	Agarose-IEF, Blot
NP-LE	11	3 (27%)	7 (27%)	6 (55%)	7/9 (78%)	9 (82%)
LE	7	0 (0%)	1 (14%)	0 (0%)	2 (29%)	0 (0%)
Golombek et al. [17]			Q_{Alb} > 9,0	IgG Index > 0,60		
NP-LE	19		5 (26%)	4/7 (57%)		
LE	12		2 (17%)	3/4 (75%)		
Kelly und *Denburg* [18]			Q_{Alb} > 7,4	IgG Index > 0,70		Methode nicht angegeben
NP-LE	35		11 (31%)	6 (17%)		6/28 (21%)
Jongen et al. [19]			Q_{Alb} > 7,0	IgG Index > 0,56	IgM Index > 0,06	
NP-LE	12		2 (17%)	2 (17%)	8 (67%)	
Graef et al. [20]		> 4 Zellen/µl	Q_{Alb} > 7,4	IgG_{Loc} > 0 mg/l		Polycrylamid-IEF, CBB
NP-LE	9	2/7 (29%)	4 (44%)	3 (33%)		3 (33%)
Lean et al. [21]			Q_{Alb} > 7,0			Agarose-IEF, Blot
NP-LE	20		6 (30%)			5 (25%)
West et al. [21]			Q_{Alb} > 9,0	IgG Index > 0,60		Agarose-Elektrophorese
NP-LE	50	9 (18%)	16 (32%)	35 (70%)		26 (52%)
LE	13	2 (15%)	4 (31%)	0 (0%)		1 (8%)
Eigene Ergebnisse		< 4 Zellen/µl	Q_{Alb} > 7,4	IgG Index > 7,0	IgM Index > 0,100	Polyacrylamid-IEF, Silber
NP-LE	21	2 (10%)	4 (19%)	4 (19%)	5 (24%)	7 (33%)
LE	14	1 (7%)	0 (0%)	0 (0%)	2 (14%)	3 (21%)

[a] $Q_{Alb} = Albumin_{CSF} * 1000/Albumin_{Serum}$
[b] IgG Index = $(IgG_{CSF}/IgG_{Serum}) * (Albumin_{Serum}/Albumin_{CSF})$

Danksagung

Mit Unterstützung der Volkswagenstiftung, I-67086

Literatur

1. Boumpas DT, Austin HA, Fessler BJ, Balow JE, Klippel JH, Lockshin MD. Systemic lupus erythematosus: emerging concepts. Part 1. Ann Intern Med 1995;122:940-50.
2. Futrell N, Schultz LR, Millikan C. Central nervous system disease in patients with systemic lupus erythomatosus. Neurology 1992;42:1648-57.
3. Hay EM, Isenberg DA. Autoantibodies in central nervous system lupus. Br J Rheumatol 1993;32:329-32.
4. Bruyn GAW. Controversies in lupus: nervous system involvement. Ann Rheum Dis 1995;54:159-67.
5. Hanson VG, Horowitz M, Rosenbluth D, Spiera H, Puszkin S. Systemic lupus erythematosus patients with central nervous system involvement show autoantibodies to a 50-kD neuronal membrane protein. J Exp Med 1992;176:565-73.
6. Bonfa E, Golombek SJ, Kaufman LD, Skelly S, Weissbach H, Brot N, et al. Association bet ween lupus psychosis and ribosomal P protein antibodies. New Engl J Med 1987;317:265-71.
7. Teh L, Isenberg DA. Antiribosomal P protein antibodies in systemic lupus erythematosus. A reappraisal. Arthritis Rheum 1994;37:307-15.
8. West SG, Emlen W, Wener MH, Kotzin BL. Neuropsychiatric lupus erythematosus: A 10-year prospective study on the value of diagnostic tests. Am J Med 1995;99:153-63.
9. Tola MR, Granieri E, Caniatti L, Paolino E, Monetti C, Dovigo LP, et al. Systemic lupus erythematosus presenting with neurological disorders. J. Neurol 1992;239:61-4.
10. Kaye BR, Neuwelt MC, London SS, De Armond SJ. Central nervous system lupus erythematosus mimicking progressive multifocal leucoencephalopathy. Ann Rheum Dis 1992;51:1152-56.
11. van Dam AP. Diagnosis and pathogenesis of CNS lupus. Rheumatol Int 1991;11:1-11.
12. Zvaifler NJ, Bluestein HG. The pathogenesis of central nervous system manifestations of systemic lupus erythematosus. Arthritis Rheum 1982;25:862-6.
13. Seibold JR, Buckingham RB, Medsger jr. TA, Kelly RH. Cerebrospinal fluid immune complexes in systemic lupus erythematosus involving the central nervous system. Sem Arthr Rheumatol 1982;12:69-76.
14. Winfield JB, Shaw M, Silverman LM, Eisenberg RA, Wilson III HA, Kofler D. Intrathecal IgG synthesis and blood brain barrier impairment in patients with systemic lupus erythematosus and central nervous system dysfunction. Am J Med 1983;74:837-44.
15. Hirohata S, Hirose S, Miyamoto T. Cerebrospinal fluid IgM, IgA and IgG indexes in systemic lupus erythematosus. Their use as estimates of central nervous system disease activity. Arch Intern Med 1985;145:1843-6.
16. Ernerudh J, Olsson T, Lindström F, Skogh T. Cerebrospinal fluid immunoglobulin abnormalities in systemic lupus erythematosus. J Neurol Neurosurg Psych 1985;48:807-13.
17. Golombek SJ, Graus F, Elkon KB. Autoantibodies in the cerebrospinal fluid of patients with systemic lupus erythematosus. Arthritis Rheum 1986;29:1090-7.
18. Kelly MC, Denburg JA. Cerebrospinal fluid immunoglobulins and neuronal antibodies in neuropsychiatric systemic lupus erythematosus and related conditions. J Rheumatol 1987;14:740-4.
19. Jongen PJH, Borbooms AMth, Lamers KJB, Raes BCM, Vierwinden G. Diffuse central nervous system involvement in systemic lupus erytematosus: intrathecal synthesis of the fourth component of complement. Neurology 1990;40:1593-6.
20. Graef IT, Henze T, Reiber H. Polyspezifische Immunreaktion im ZNS bei Autoimmunerkrankungen mit ZNS-Beteiligung. Z ärztl Fortbild 1994;88:113-7.
21. Mc Lean BN, Miller D, Thompson EJ. Oligoclonal banding of IgG in CSF, blood-brain-barrier function, and MRI findings in patients with sarcoidosis, systemic lupus erythematosus, and Behcet's disease involving the nervous system. J Neurol Neurosurg Psych 1995;58:548-55.

Usage of Consensus Polymerase Chain Reaction (PCR) Assays from Variable Ribosomal Regions for the Detection of Bacterial Infections of the Human Central Nervous System*

Einsatz variabler, ribosomaler Genregionen in einer Konsensus Polymerase Chain Reaction (PCR) zum Nachweis bakterieller Infektionen im humanen Nervensystem*

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Keywords: Polymerase Chain Reaction; Meningitis, Bacterial/cerebrospinal fluid; RNA, Bacterial/genetics; RNA, Ribosomal, 23S/genetics; Cerebrospinal Fluid; Sequence Analysis, DNA.

Schlüsselwörter: Polymerase Kettenreaktion; Meningitis: bakterielle/Liquor cerebrospinalis; RNA, Bakterielle/Genetik; RNA, Ribosomale, 23S/Genetik; Sequenzanalyse, DNA.

The time required to obtain a positive culturing result from the cerebrospinal fluid (CSF) in patients with a bacterial meningitis or meningoencephalitis lasts at least an 8-20 h incubation period in culture medium followed by biochemical and immunological tests to identify the bacteria. In cases of infections with slowly growing organisms or with low bacterial counts this time period might even be longer. Latex agglutination tests have a low sensitivity and demonstrate reliable results only for samples with over 10^5 CFU per ml [1].

However, because of the high mortality rate being associated with untreated bacterial meningitis or meningoencephalitis, antibiotic therapy is mostly prescribed empirically. This procedure promotes the unselected usage of antibiotics and supports the increasing risk of the development of resistant pathogens.

Assays based on the polymerase chain reaction (PCR) technology have the potential for greater sensitivity and higher rapidity. Although PCR has been applied for numerous pathogens there are some bacteria being a relevant cause of meningitis for which there is no valide PCR assay published. In addition, it is rather laborious and inefficient to pipette twenty or more different PCR assays to the diagnose of one potential bacterial infection. In the case that the PCR reaction is negative the investigator will be always uncertain whether there is really no bacterial nucleic acid present in the sample or whether it is a pathogen for what he has no assays established in the laboratory. The use of universal PCR primers targeting highly conserved regions in a broad range of different bacteria might solve these problems [2].

Molecular analysis of ribosomal DNA sequences has proved extremely valuable for taxonomy, via sequence comparison, for epidemiologic investigation of microorganism, via conventional ribotyping [3] and for diagnostic procedures. Species-specific sequence variants can be detected in PCR-amplified fragments of the 16S-23S [4, 5] or the 23S-5S rRNA [6] intergenic spacer region. As an example we have previously described the usage of the 23S-5S-spacer region for the simple molecular differentiation of members of the *Legionellae* family [7], a method that uses conserved PCR primers located within the 23S and 5S ribosomal DNA genes to differentiate strains of bacteria on the basis of sequence variations in the intergenic spacer region of the ribosomal RNA operon. *Greisen* and colleagues [8] has published a similar approach for the detection of bacteria in the CSF by using the 16 rRNA gene for the differentiation of bacterial species. However the authors have the problem to detect single strains from one species or even some bacterial species because the 16S gene exhibits only some heterogenous loci which are difficult for being usable in one assay format.

Here we describe the PCR procedure for the identification of a number of pathogens being responsible for bacterial meningitis or meningoencephalitis using the 23S-5S intergenic spacer region in a universal consensus PCR.

As an example for the sequencing results as well as

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* Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic IV, Marburg an der Lahn, September 29 and 30, 1995

Nonstandard abbreviations: CFU, colony-forming units; CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

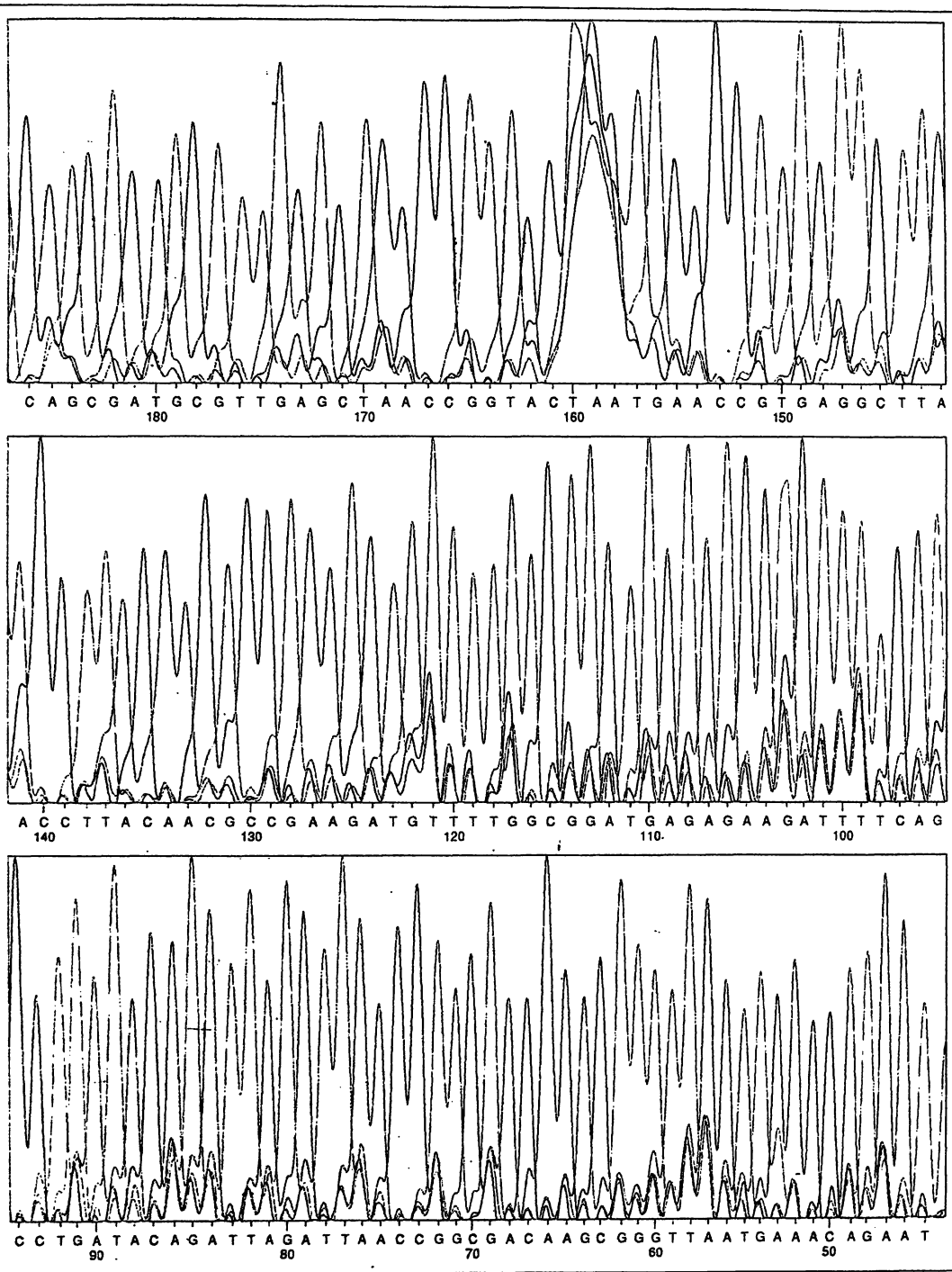

Figure 1 Demonstration of the sequencing data of the plus-strand of the *E. coli* (ATCC 20922) intergenic 23S-5S spacer fragment using the ALF-Pharmacia sequencing machine. The four different coloured peaks represent the four different nucleotides (A, C, G, T). The detailed experimental procedures regarding the isolation of bacterial DNA, the amplification of the 23S-5S-spacer region as well as the sequencing experiments were described elsewhere from our group [6, 9]. The 23S and 5S primers were designed on the basis of a consensus of all available 23S and 5S sequences published in EMBL (primer *79L* and *82R*). The 23S primer was supplied with a M13 sequence at its 5' terminal, the 5S primer with a rM13 sequence; this allows the use of M13 and rM13 universal sequencing primers [11]. DNA sequencing was performed as previously described [11]. For each isolate the (+) and the (-) strand were analyzed separately to ensure accuracy.

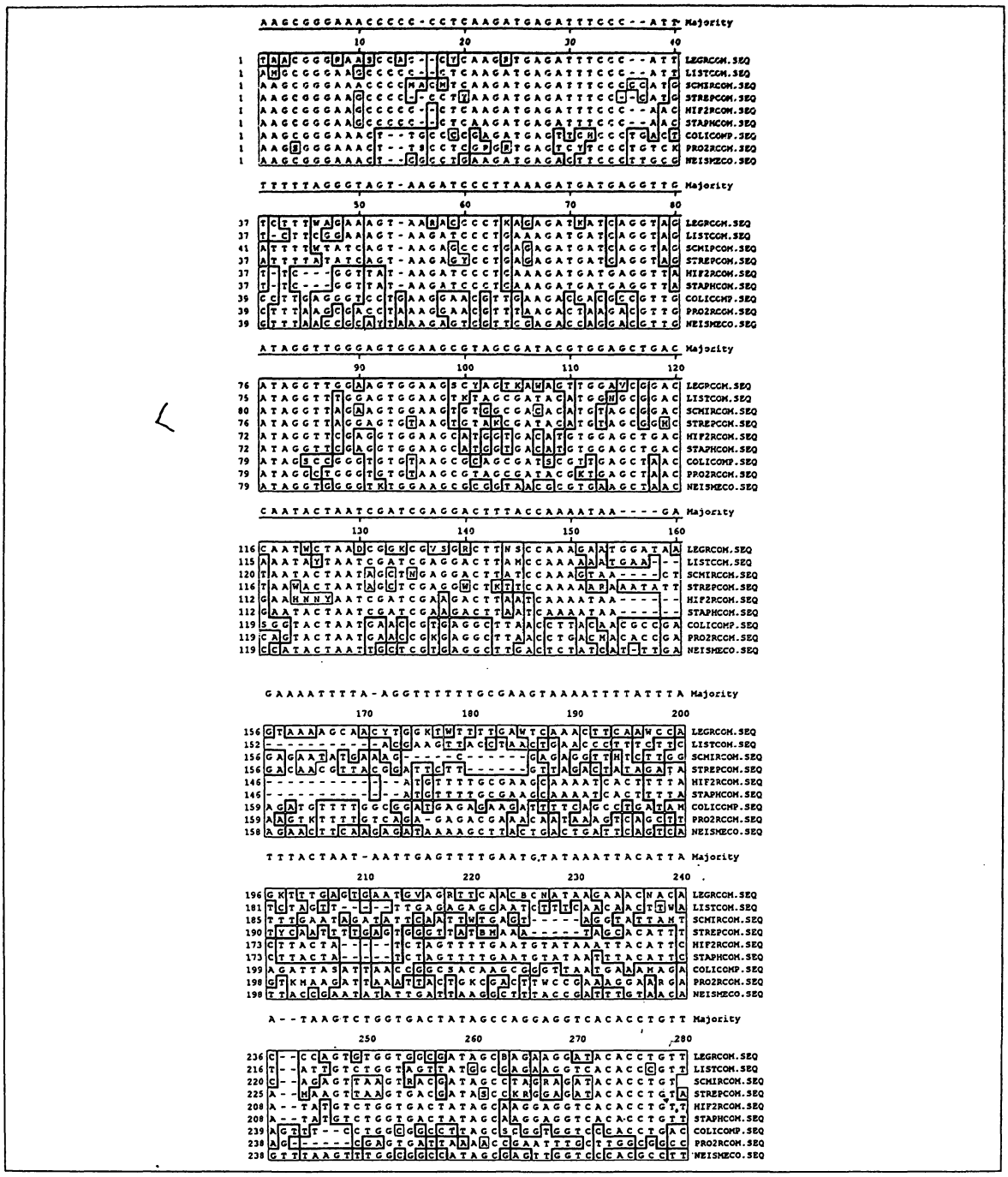

Figure 2 Sequence alignment of the 3' terminus of 23S rDNA, 23S-5S spacer region, and the 5' portion of 5S rDNA sequences of nine different bacterial species. The DNA extraction from *Escherichia coli* (colicomp, ATCC 20922), *Staphylococcus aureus* (staphcom, ATCC 33589), β-hemolysing *Streptococcus* (strepcom, ATCC 33400), *Listeria monocytogenes* (listcom, ATCC 15313), *Neisseria meningitidis* (neismeco, ATCC 13077), *Legionella pneumoniae* (legrcom, ATCC 33152), *Proteus mirabilis* (pro2rcom, ATCC 29906), *Streptococcus mitis* (scmir com, ATCC 33399), *Haemophilus influenzae* (hif2rcom, ATCC 33391) was done according a standard phenol chloroform extraction method [10]. A 50 μL amplification reaction was set up for each DNA sample to be analyzed. DNA was added to the amplification reaction containing PCR buffer with 1,5mmol/l $MgCl_2$, 200 μmol/l dNTPs, 0.2 μmol/l of each primer and 1 U Taq polymerase (AmpliTAQ, Perkin Elmer Cetus, USA). An initial denaturation of 5 min at 95 °C, 35 cycles (95 °C, 40s; 50 °C, 60s; 72 °C, 60s), and a final elongation step of 5 min at 72 °C were performed. All mismatches in the alignment are boxed. The statistical analysis of the sequences was done by the modified Maximum-Likelihood method.

sequence of the intergenic spacer region flanked by the 23S gene (on the left) and the 5S gene (on the right).

We demonstrate the 23S-5S intergenic sequence of nine different bacteria that are often the causes of meningitis or meningoencephalitis (fig. 2). All specimens could be amplified using the same conditions and primers. The resulting sequence consists of three domains: the conserved 3' region of the 23S rDNA (position 1 to 130), the complete 23S-5S spacer region (position 131 to 250), and the conserved 5' region of the 5S rDNA (fig. 2). In almost all cases an unambiguous sequence could be obtained; ambiguities were noted at certain positions for some species. In some cases the ambiguity might be due to microheterogeneity among the several copies of the rRNA operon, as signals for two bases were consistently observed at a few given positions in multiple sequencing experiments.

The length of the sequences varied from 269bp to 322bp. As expected, sequences in the 23S and 5S regions were rather conserved among bacteria which are closely related in a phylogenetic manner (e.g. *Streptococcus pneumoniae* and *Streptococcus mitis*). Between different species, like *Listeria monocytogenes* and *Proteus mirabilis*, the 23S-5S spacer region demonstrated highly variable sequences, and was responsible for the observed length variation of the PCR amplicons.

The analyzed sequence data now allow to create specific probes for reverse dot-blotting the amplification product with the aim of detecting specifically the different bacteria. We are able to design a probe to the 5S region detecting all Gram negative and Gram positive bacteria which could be used as a screening system. Species-specific probes were designed to hybridize to a portion of the 23S-5S spacer region which is unique to this species. This method allows the use of just one amplicon (what means only one PCR assay) to detect a broad spectrum of different bacteria within the CSF.

The 23S-5S reverse-dot blot method presented here may possess advantages compared with 16S-23S systems. The 23S-5S spacer region is smaller than the 16S-23S spacer; therefore, a PCR amplification system spanning the complete 23S-5S-spacer region is expected to be more sensitive than the 16S-23S-region since the amplification efficiency of smaller fragments tends to be higher than that of larger PCR products. In addition, the use of an amplicon containing both the 23S-5S spacer region and a portion of the 5S rRNA coding sequence allows simultaneous genus- and species-specific detection.

In summary, the intergenic ribosomal sequences described allow the identification of the most frequent causes of bacterial meningitis and can form the basis of a more rapid and sensitive means of detecting bacteria in CSF. The use of a panel of different probes designed with the sequences of the spacer region is suited to a detection format in which the probes are immobilized on a solid phase (e.g. ELISA, membranes) [7]. This format enables the use of a single CSF sample to obtain multiple probe hybridization results.

References

1. Bingen E, Lambert-Zechovsky N, Mariani-Kurkdjian P, Doit C, Aujard Y, Fournerie F, et al. Bacterial counts in cerebrospinal fluid of children with meningitis. Eur J Clin Microbiol Infect Dis 1990;9:278-81.
2. Wilson K, Blitchington R, Greene R. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. J Clin Microbiol 1990;28:1942-6.
3. Cartwright C, Stock F, Beekmann SE, Williams EC, Gill VJ. PCR amplification of rRNA intergenic spacer regions as a method for epidemiologic typing of *Clostridium difficile*. J Clin Microbiol 1995;33:184-7.
4. Glennon M, Smith T, Cormican M, Noone D, Barry T, Maher M, et al. The ribosomal intergenic spacer region: a target for the PCR based diagnosis of tuberculosis. Tubercle Lung Dis 1994;75:353-60.
5. Bourque SN, Valero JR, Lavoie MC, Levesque RC. Comparative analysis of the 16S to 23S ribosomal intergenic spacer sequences of *Bacillus thuringiensis* strains and subspecies and of closely ralated species. Appl Environ Microbiol 1995;61:1623-6.
6. Heidrich B, Tiecke F, Robinson PN, Steinhoff D, Fehrenbach FJ, Rolfs A. Automated direct sequencing of Legionella 5s rDNA. Med Microbiol Lett 1994;3:279-90.
7. Robinson PN, Heidrich B, Tiecke F, Fehrenbach F, Rolfs A. Detection of *Legionellae* with PCR and Reverse Dot-blot of the 23S-5S rRNA Spacer Region. FEBS Microbiol 1996 in press.
8. Greisen K, Loeffelholz M, Purohit A, Leong D. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in the cerebrospinal fluid. J Clin Microbiol 1994;32:335-51.
9. Heidrich B, Robinson PN, Tiecke F, Steinhoff D, Fehrenbach FJ, Rolfs A. Genetische Verwandtschaft innerhalb des Genus Legionella - DNS Sequenzuntersuchungen an ribosomalen Genen. Klin Labor 1994;40:211-16.
10. Rolfs A, Schuller I, Finckh U, Weber-Rolfs I. PCR: Clinical diagnostics and research. Berlin, Heidelberg, New York: Springer, 1992:82-89.
11. Rolfs A, Weber, I. Fully-automated, non-radioactive solid-phase sequencing of genomic DNA obtained from polymerase chain reaction. BioTechniques 1994;17:782-7.

Clinical Significance of Immunoblotting of IgG Oligoclonal Bands with Respect to Specific Antigens*

Klinische Bedeutung des Immunblotting von oligoklonalen Banden in bezug auf spezifische Antigene*

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Keywords: Antibodies, Bacterial/cerebrospinal fluid; Antibodies, Viral/cerebrospinal fluid; IgG/cerebrospinal fluid; Immunoblotting.

Schlüsselwörter: Antikörper, Bakterielle/Liquor cerebrospinalis; Antikörper, Virale/Liquor cerebrospinalis; IgG/Liquor cerebrospinalis; Immunblotting.

The presence of oligoclonal immunoglobulin G (IgG) bands restricted to the cerebrospinal fluid (CSF) is the hallmark of the intrathecal humoral immune response observed in a very high proportion (95%) of patients with Multiple Sclerosis [1], but also in chronic infections of the central nervous system (CNS) [2]. The determination of the antibody activity of these IgG bands is clinically relevant, especially from a diagnostic point of view. The antigen-mediated capillary blot technique, first described by Dörries et al [3], is a powerful tool for characterizing the antibody specificity of these bands and has now supplanted the imprint electro-immunofixation method [4].

This blot technique allows the unambiguous detection of an intrathecal synthesis of specific antibodies in various infections of the CNS by using a minimal amount (10 μl) of CSF samples. The simultaneous analysis of the corresponding serum makes possible the discrimination between a local synthesis and a passive transfer of specific antibodies across the blood-CSF barrier. A passive transfer accounts for a „mirror pattern". A direct comparison between the total IgG pattern and the specific IgG antibodies is also made possible by this technique. Specific oligoclonal antibodies may be present in the absence of detectable oligoclonal IgG bands. This technique is therefore a diagnostic tool in demonstrating the CNS involvement by various infectious agents.

These oligoclonal antibodies (mainly IgG, more rarely IgA) are indeed observed in various chronic infections of the CNS: neurobrucellosis, neuroborreliosis, tuberculous meningitis, herpetic meningitis and/or encephalitis, varicella zoster meningitis or meningoradiculitis, in utero cytomegalovirus infection, neuro-AIDS, HTLV-1 associated myelopathy (table 1).

However, this technique also has some limitations:

- results are obtained by the visual inspection (eye reading) of the immunoblots, and only semi-quantitative data may be obtained by scanning densitometry;
- complex antigenic mixtures were used in most cases and only antibodies directed against the most abundant antigenic determinants are likely detected;
- the occurrence of crossed-reactions must be taken into account, for example between Herpes simplex and varicella zoster viruses, between Borrelia and Treponema;
- the occurrence of polyspecific B-cell activation and hence, the production of antibodies to unrelated antigens must be recognized. This polyspecific reaction is frequent in Multiple Sclerosis: 70% of these patients display oligoclonal anti-measles IgG antibodies in their CSF, 60% anti-rubella antibodies, 40% anti-varicella zoster antibodies and 30% anti-mumps antibodies [9].

In the future, purified immunodominant antigen(s) for the coating of the blot (for example, recombinant antigens) should be used as often as possible. A correlation between the affinity blot technique and the calculation of an antibody specific index (ASI) should be done for each antigen studied. The search for autoantibodies against CNS antigens could be also performed by means of this technique.

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Nonstandard abbreviations: AIDS, aquired immunodeficiency syndrome; CNS, central nervous system; CSF, cerebrospinal fluid; HTLV, human T-cell lymphotropic virus; IEF, isoelectric focusing; IgG, immunoglobulin G.

Table 1 The antigen-mediated capillary blot technique as a tool for the detection of CSF-specific oliqoclonal antibodies (personal results)

Infectious agent	Antigens	References
Brucella abortus	purified lipopolysaccharides	[5]
Borrelia burgdorferi	crude preparation (home-made)	[6]
Mycobacterium tuberculosis	crude preparation (H37Ra)	[7]
Herpes simplex	crude preparation commercially available	[8]
Varicella zoster	crude preparation commercially available	[9]
Mumps	crude preparation commercially available	[9]
Measles	crude preparation commercially available	[9]
Rubella	crude preparation commercially available	[9]
Cytomegalovirus	crude preparation commercially available	[9]
Human immunodeficiency virus	crude preparation (home-made)	[10]

References

1. Sindic CJM, Laterre EC. Oligoclonal free kappa and lambda bands in the cerebrospinal fluid of patients with multiple sclerosis and CNS infections. An immunoaffinity-mediated capillary blot study. J Neuroimmunol 1991;33:63-72.
2. Laterre EC, Callewaert A, Heremans JF, Sfaello Z. Electrophoretic morphology of gamma globulins in cerebrospinal fluid of multiple sclerosis and other diseases of the nervous system. Neurology 1970;20:982-90.
3. Dorries R, Ter Meulen V. Detection and identification of virus-specific, oligoclonal IgG in unconcentrated cerebrospinal fluid by immunoblot technique. J Neuroimmunol 1984;7:77-89.
4. Vandrik B, Nordal HJ, Vartdal F, et al. Imprint electroimmunofixation of antibodies separated by agarose electrophoresis or by electrofocusing. In: Axelsen N, editor. Handbook of Immunoprecipitation-in-Gel Technique. Scand J Immunol 1981;Suppl 10.
5. Bukasa K, Limet J, Laterre EC, Sindic CJM. Determination of the antibody activity of CSF oligoclonal bands by immunoblotting. In: Poeck K, Hacke W, Schneider R, editors. Verhandlungen der Deutschen Gesellschaft für Neurologie. Berlin (Deutschland): Springer, 1987;4:405-7.
6. Depre A, Sindic CJM, Bukasa K, Bigaignon G, Laterre EC. Formes encéphalomyélitiques de l'infection à Borrelia Burgdorferi. Revue Neurol 1988;144:416-20.
7. Sindic CJM, Boucquey D, Van Antwerpen MP, Baelden MC, Laterre EC, Cocito C. Intrathecal synthesis of anti-mycobacterial antibodies in patients with tuberculous meningitis. An immunoblotting study. J Neurol Neurosurg Psychiatry 1990;53:662-6.
8. Boucquey D, Chalon MP, Sindic CJM, Lamy ME, Laterre EC. Herpes simplex virus (HSV) type II meningitis without genital lesions:an immunoblot study. J Neurol 1990;237:285-9.
9. Sindic CJM, Monteyne P, Laterre EC. The intrathecal synthesis of virus- specific oligoclonal IgG in Multiple Sclerosis. J Neuroimmunol 1994;54:75-80.
10. Bukasa K, Sindic CJM, Bodeux M, Burtonboy G, Laterre EC. Sonnet J. Anti-HIV antibodies in the CSF of AIDS patients. A serological and immunoblotting study. J Neurol Neurosurg Psychiatry 1988;51:1063-8.

Polymerase Chain Reaction as a Tool for Rapid Diagnosis of Meningitis and Encephalitis*

Polymerase Chain Reaction, eine Technik zur schnellen Diagnose von Meningitis und Encephalitis*

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Keywords: DNA Viruses/cerebrospinal fluid; Leukencephalopathy, Progressive Multifocal/diagnosis; Polymerase Chain Reaction; RNA Viruses/cerebrospinal fluid; Tuberculosis, Meningeal/diagnosis.

Schlüsselwörter: Polymerase Kettenreaktion; DNA Viren/Liquor cerebrospinalis; RNA Viren/Liquor cerebrospinalis; Leukenzephalopathie, progressive multifokale/Diagnostik; Tuberkulose, meningeale/Diagnostik.

Polymerase chain reaction (PCR) technique can be used to amplify any genome as far as the gene sequences are known. In this short review, we shall discuss the application of this technique to detect mycobacteria and viruses in the cerebrospinal fluid (CSF).

Mycobacteria

The diagnosis of tuberculous meningitis is often difficult, uncertain and slow. The 'gold standard' remains the identification of *M. Tuberculosis* in the CSF by direct staining or by culture. Protein and glucose levels may be normal at the first CSF analysis and acid-fast bacilli rarely are directly detectable [1]. In the last five years, a few papers have been devoted to the diagnosis of tuberculous meningitis by PCR amplification in CSF. There are some discrepancies in these studies, the major problem being the detection of false positive results, especially with some sequences. The sensitivity is particularly high with the 'nested' amplification procedure [2]. In a near future, such a hopeful technique will probably provide a diagnosis of tuberculous meningitis very earlier than the conventional bacteriological cultures.

RNA Virus

RNA viruses are more difficult to detect by PCR than DNA viruses. RNA must first be transformed in cDNA enzymatically before any amplification, and RNA is less stable than DNA. Several laboratories are adapting PCR to the diagnosis of 'benign lymphocytic meningitis' caused by enteroviruses. Commercial PCR assays are under development, and large scale studies are under progress. It may be possible to identify the serotype of the enterovirus by analyzing the amplification products with restriction enzymes [3].
A particular case is represented by the poliovirus. Possible viral persistence in the central nervous system (CNS) of patients with the postpolio syndrome could be detected by PCR. So far, there is no consensus on this question and further studies are still needed [4].
PCR has also been applied to detect human immunodeficiency virus (HIV) genome in the CSF of AIDS patients with high sensitivity. There was no correlation with the clinical stage or the neurological symptoms [5].

DNA Virus

PCR is particularly useful for the diagnosis of herpetic CNS infections including agents such as cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), and Herpes simplex virus (HSV). A recently published study about the diagnosis of cytomegalovirus infection in the CNS of AIDS patients showed that PCR had a higher specificity in the acute phase than intrathecal antibody detection [6]. PCR has also been applied to show that acute aseptic meningitis could be due to varicella-zoster virus even in the absence of cutaneous lesions [7]. The detection of Epstein-Barr virus genome in the CSF by PCR has

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Nonstandard abbreviations: AIDS, aquired immunodeficiency syndrome; CNS, central nervous system; CSF, cerebrospinal fluid; HSV, herpes simplex virus; PCR, polymerase chain reaction.

been correlated to the diagnosis of AIDS-related primary lymphoma of the CNS [8].

For the moment, the largest published studies using PCR in CNS infections concern Herpes simplex virus (HSV) type 1 and 2 encephalitis and meningitis. High sensitivity and specificity have been shown with type-specific [9,10], and group-specific [11] techniques. In our hands, a time-relation between PCR and the detection of specific HSV oligoclonal bands by immunoaffinity-mediated capillary blotting makes these two techniques useful and complementary. PCR is known to be classically positive at the onset and HSV DNA usually persists in the CSF until the fifth day following the start of the therapy, preceding the detection of intrathecal production of specific antibodies by 7-10 days. The post-treatment CSF sample is generally negative for HSV DNA, while intrathecal synthesis of specific antibodies must confirm the diagnosis. Using these two methods, we were also able to demonstrate the HSV type 2 etiology in a case of recurrent meningitis and encephalitis [12].

An additional field of possible PCR application is the diagnosis of progressive multifocal leukoencephalopathy by amplification of the JC DNA in the CSF [13].

Conclusion

All these examples show how wide the domain is of application of PCR and how promising the results are so far. However, those results, for most of the infectious agents, are still limited. Moreover, one should be aware that this technique is still delicate and requires quality controls.

Aknowledgements

The authors are members of the European Union Concerted Action on Virus Meningitis and Encephalitis. We are thankful to Prof. P. L. Masson for careful reading of the manuscript.

References

1. Monteyne P, Sindic CJM. The diagnosis of tuberculous meningitis. Acta Neurol Belg 1995;95:80-7.
2. Liu PY, Shi Z, Lau Y, Hu B. Rapid diagnosis of tuberculous meningitis by simplified nested amplification protocol. Neurology 1994;44:1161-4.
3. Kämmerer U, Kunkel B, Korn K. Nested PCR for specific detection and rapid identification of human picornaviruses. J Clin Microbiol 1994;32:285-91.
4. Sharief. Poliovirus persistence in the postpolio syndrome. Ann Neurol 1993;34:415-6.
5. Chiodi F, Keys B, Albert J, Hagberg L, Lundeberg J, Uhlen M, et al. Human immunodeficiency virus type 1 is present in the cerebrospinal fluid of a majority of infected individuals. J Clin Microbiol 1992;30:1768-71.
6. Weber T, Beck R, Stark E, Gerhards J, Korn K, Haas J, et al. Comparative analysis of intrathecal antibody synthesis and DNA amplification for the diagnosis of cytomegalovirus infection in the central nervous system in AIDS patients. J Neurol 1994;241:407-14.
7. Echevarria JM, Casas I, Tenorio A, de Ory F, Martinez-Martin P. Detection of varicella-zoster virus-specific DNA sequences in cerebrospinal fluid from patients with acute aseptic meningitis and no cutaneous lesions. J Med Virol 1994;43:331-5.
8. Cinque P, Brytting M, Vago L, Castagna A, Parravicini C, Zanchetta N, et al. Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. Lancet 1993;342:398-401.
9. Aurelius E, Johansson B, Sköldenberg B, Staland A, Forsgren M. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. Lancet 1991;33:189-92.
10. Aurelius E, Johansson B, Sköldenberg B, Forsgren M. Encephalitis in immunocompetent patients due to herpes simplex type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. J Med Virol 1993;39:179-86.
11. Rozenberg F, Lebon P. Amplification and characterization of herpes virus DNA in cerebrospinal fluid from patients with acute encephalitis. J Clin Microbiol 1991;29:2412-7.
12. Monteyne Ph, Sindic CJM, Laterre EC. Recurrent meningitis and encephalitis associated with herpes simplex type 2: demonstration by polymerase chain reaction. Eur Neurol 1996;36:176-7.
13. Weber T, Turner RW, Frye S, Ruf B, Haas J, Schielke E, et al. Specific diagnosis of progressive multifocal leukoencephalopathy by polymerase chain reaction. J Infect Dis 1994;169:1138-41.

D-Lactate and L-Lactate Production by Bacteria Causing Meningitis and Sepsis*

D-Lactat- und L-Lactat-Bildung von Bakterien, die Meningitis und Sepsis hervorrufen*

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Keywords: Bacteria/metabolism; Gram-Negative Bacteria; Gram-Positive bacteria; Lactates/cerebrospinal fluid; Meningitis, Bacterial/diagnosis; Sepsis.

Schlüsselwörter: Bakterien/Stoffwechsel; Gram-negative Bakterien; Gram-positive Bakterien; Lactat/Liquor cerebrospinalis; Meningitis, bakterielle/Diagnostik; Sepsis.

In oxygen-limited, or anaerobic cultures, many kinds of microorganisms capable of carbohydrate fermentation, accumulate extracellular lactic acid. D-lactate is produced only by some of the procaryotic organisms. It cannot be utilized by mammalian tissues [1-4]. Therefore, for the purpose of food production, lactic acid bacteria producing L-lactate alone have been selected [1]. After intestinal resorption of D-lactate from alimentary soucres, or produced by members of the indigenous bacterial flora, low levels of D-lactate may be present in the human blood and tissues [3-9]. Levels of D-lactate exceeding this background may be accumulated in the course of systemic infections by the catabolic activities of invasive bacteria [2-4,6,10]. However, our knowledge of the occurence of catabolic D-lactate in various bacterial groups causing invasive infections is far from complete. The aim of our present study was to gain an idea of the capabilities of bacterial taxa causing sepsis and meningitis to produce D-lactate *in vitro*.

Under the conditions of statically incubated (i.e. oxygen-limited) late exponential cultures in standardized PYG medium [11], significant concentrations of lactate were accumulated by members of Gram-positive and Gram-negative bacterial groups that are capable of fermentative glycolysis (fig. 1). Cultures of organisms producing acid from glucose only by oxidation, or not at all, did not contain more than trace amounts of lactate, if any [12].

Among the facultatively anaerobic, fermentative Gram-negative rods, members of the family *Enterobacteriaceae* were, in general, strong D-lactate and in some cases moderate L-lactate producers. Members of the family *Pasteurellaceae* reached lower growth yields in PYG medium and accumulated moderate amounts of D-lactate. Non-fermentative Gram-negative organisms did not produce lactate [12].

Among the Gram-positive glucose fermenters, *Enterococcus faecalis* and *faecium, Staphylococcus aureus* and other *Staphylococcus* spp., proved to be strong to moderate D- and L-lactate producers (with different individual patterns; [cf. 14]). With our test conditions, members of the genus *Streptococcus* produced only small amounts of L-lactate and no D-lactate [12].

So far, anaerobic bacterial groups that may contain D-lactate producers were not investigated.

The data presented indicate that several important groups of organisms causing sepsis and meningitis [13] are strong D-lactate producers, but other taxa of similar clinical importance do not accumulate D-lactate levels *in vitro*, under the experimental conditions of this study.

Since the production of lactate and its racemation greatly varies with the culture conditions, in particular, medium composition and degree of anaerobiosis, we cannot expect that *in vitro* findings will be quantitatively reproduced under the conditions prevailing in the infected host's tissues. First results of ongoing investigations seem to corroborate the assumption that lactate production *in vivo* qualitatively corresponds to that observed by *in vitro* experiments.

We can expect that organisms producing D-lactate *in vitro* will also do so *in vivo*, but this question must be answered by careful analysis of clinical cases [6]. At present we feel justified in stating that in accordance with previous investigators [2,6,10], elevated D-lactate in body fluids can only be explained as a specific bacterial product, whereas, on the other hand, in the absence of elevated D-lactate concentrations invasive bacterial and mycotic infections cannot be ruled out.

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Nonstandard abbreviations: PYG medium, peptone-yeast-glucose medium; sp., species; spp., subspecies.

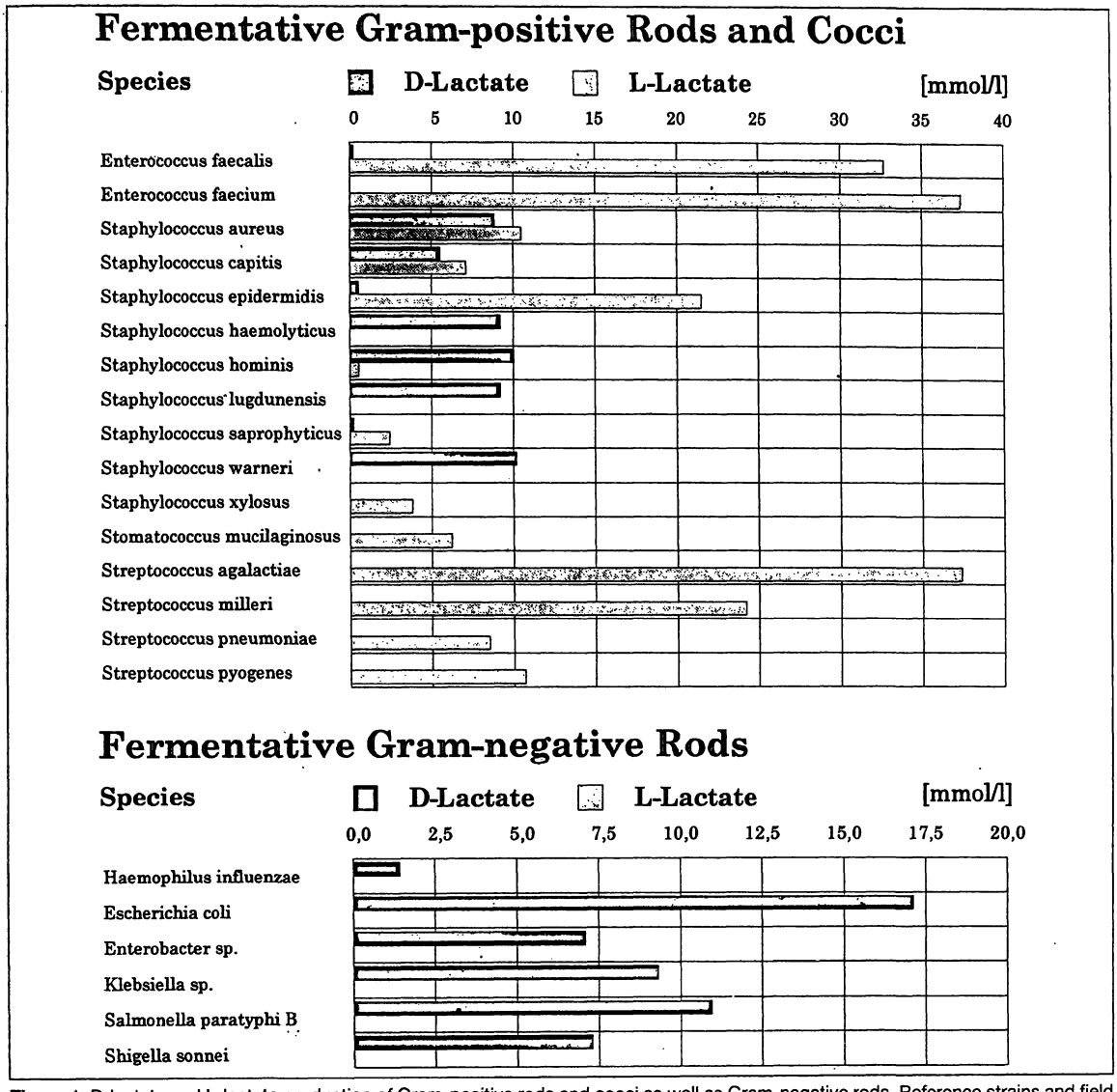

Figure 1 D-lactate and L-lactate production of Gram-positive rods and cocci as well as Gram-negative rods. Reference strains and field isolates of bacterial species frequently causing sepsis and meningitis were propagated in oxygen-limited, or anaerobic peptone-yeast-extract-glucose (PYG) medium [11]. The cell-free supernatants (sterile filtrates) of late exponential cultures were examined for D(-)-lactate and L(+)-lactate concentrations (mmol/l supernatant) at least in duplicate using stereospecific lactate dehydrogenase reactions performed photometrically with Boehringer Mannheim tests [6].

References

1. Drury DR, Wick AN. Chemistry and metabolism of L(+) and D(-) lactic acids. Ann N Y Acad Sci 1965;119:1061-9.
2. Marcos MA, Vila J, Gratacos J, Brancos MA, Jimenez de Anta MT. Determination of D-lactate concentration for rapid diagnosis of bacterial infections of body fluids. Eur J Clin Microbiol Infect Dis 1991;10:966-9.
3. Smith SM, Eng RHK, Campos JM, Chmel H. D-lactic acid measurements in the diagnosis of bacterial infections. J Clin Microbiol 1989;27:385-8.
4. Smith SM, Eng RHK, Buccini F. Use of D-lactic acid measurements in the diagnosis of bacterial infections. J Infect Dis 1986;154:658-64.
5. Carr DB, Shih VE, Richter JM, Martin JB. D-Lactic acidosis simultating a hypothalamic syndrome after bowel bypass. Ann Neurol 1982;11:195-7.
6. Kleine TO. D-lactate and L-lactate in cerebrospinal fluid with acute inflammatory diseases of the central nervous system. Lab med 1991;15:114-6.
7. Oh MS, Phelps KR, Traube M, Barbosa-Saldivar JL, Boxhill C, Carroll HJ. D-lactic acidosis in a man with short bowel syndrome. N Engl J Med 1979;301:249-52.
8. Schoorel EP, Giesberts MAH, Blom W, van Gelderen HH. D-lactic acidosis in a boy with short bowel syndrome. Arch Dis Child 1980;55:810-2.
9. Stolberg L, Rolfe R, Gitlin N, Merri J, Mann L, Linder J, et al. D-lactic acidosis due to abnormal gut flora. Diagnosis and treatment in two cases. N Engl J Med 1982;306:1344-8.
10. Komorowski RA, Farmer SG, Knox KK. Comparison of cerebrospinal fluid C-reactive protein and lactate for diagnosis of meningitis. J Clin Microbiol 1986;24:982-5.
11. Phillips E, Nash P. Culture media. In: Lennette EH et al. editors. Manual of Clinical Microbiology. 5th edition. Washington D.C.: Am Soc Microbiol 1985:1051-92.
12. Janke KH, Kleine TO, Nebel P, Mannheim W. Bildung von D-Lactat und L-lactat durch bakterielle Meningitis- und Sepsis-Erreger. In: Günther W, Hempel V, Wulff G, editors. InCom'96. International Symposium on Instrumentalized Analytical Chemistry and Computer Technology. Moers(Deutschland): InCom-Bureau IAS 1996:406.
13. Geerdes-Fenge HF, Lode H, Ziegler D, Hund M, Wagner J, Fangmann W, et al. Erregerspektrum der Sepsis. Chemotherapie J 1994;3:131-43.
14. Holt JG et al. editors. Bergey's Manual of Determinative Bacteriology. 9th edition. Baltimore (USA): Williams & Wilkins, 1994.

D-Lactate and L-Lactate versus Free Bacterial Antigens in Cerebrospinal Fluid (CSF) with Different Forms of Meningitis*

D-Lactat und L-Lactat versus freie Bakterien-Antigene im Liquor cerebrospinalis bei verschiedenen Formen von Meningitis*

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Keywords: Antigens, Bacterial/cerebrospinal fluid; Cerebrospinal fluid; Glucose/cerebrospinal fluid; Lactates/cerebrospinal fluid; Leukocytes/cerebrospinal fluid; Meningitis, Bacterial/diagnosis, Meningitis, Viral/diagnosis.

Schlüsselwörter: Antigene, bakterielle/Liquor cerebrospinalis; Liquor cerebrospinalis; Glucose/Liquor cerebrospinalis; Leukozyten/Liquor cerebrospinalis; Meningitis, bakterielle/Diagnostik; Meningitis, virale/Diagnostik.

In 1979 we showed in a multicentre study that *L-lactate* content, measured enzymatically [1], proved to be the best in differential diagnosis between acute bacterial and abacterial (viral) meningitis evaluating white cell count, total protein, L-lactate dehydrogenase and L-lactate: The cut-off value of 3.5 mmol/l L-lactate brought a sensitivity of 100% [1,2]. However, determination of L-lactate concentration alone led to misdiagnosis of acute bacterial meningitis with patients suffering from brain tumour, acute stroke, CNS intoxication and subarachnoidal or intracerebral hemorrhages [1-4]. Low *glucose* contents (< 49 mg/dl) in lumbar CSF were uncertain in discrimination of these diseases [2-4].

The diagnostic combination of lactate content ≥ 3.5 mmol/l with *leukocyte count* ≥ 800 M/l improved specificity of the lactate test to 99% and the predictive value to 88% [1,2]. Thus brain tumours, acute strokes and CNS intoxications were excluded as well as cerebrovascular diseases. Further improvement in differentiation brought an immune nephelometric test for *lysozyme* (for acute bacterial meningitis cut off ≥ 3 mg/l) [2] and the combination of *erythrocyte count* ≥ 2,100 M/l [2] with lactate content ≥ 3.5 mmol/l for the defination of cerebrovascular diseases in CSF [5]. However, in treated cases of bacterial meningitis lysozyme content fell to viral or lymphocyte-meningitis levels and lactoferrin ones or polymorphonuclear leukocyte elastase complex levels proved to be not helpful in discrimination of these meningitis forms [5].

Detection of free *bacterial antigens* with manual latex tests in CSF yielded a diagnostic sensitivity of 80 to 100% with acute bacterial meningitis [6]. Thus positive detection of bacterial antigens together with lactate levels ≥ 3.5 mmol/l confirm a bacterial meningitis even in treated cases with sterile cultures [6]. With treated cases latex tests showed a diagnostic sensitivity of < 50%. With negative latex tests and L-lactate levels ≥ 3.5 mmol/l a treated bacterial meningitis is indicated if leukocyte counts were > 800 M/l [6]. Comparison between manual latex tests and Particle Counting Immunoassays (PACIA) proved the latter to be most sensitive in detection of free bacterial and fungal antigens in CSF; it was even more sensitive than EIA and ELISA [7].

D-lactate, a specific metabolite of some bacteria [8], was useful in diagnosis of meningitis [9]. Therefore, we compared the detection of 8 bacterial and fungal antigens by PACIA with D-lactate levels in CSF from 89 patients with meningitis, treated and untreated, all yielding negative antigen results; the upper limit of D-lactate was 0.18 mmol/l (table 1). Therefore, the cut off of D-lactate was set up to ≥ 0.20 mmol/l to diagnose a bacterial meningitis in CSF [9]. D-Lactate levels > 0.20 mmol/l were found in 12 cases of bacterial meningitis untreated and in some of 18 treated cases

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Nonstandard abbreviations: CSF, cerebrospinal fluid; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; PACIA, particle counting immunoassay; spp., subspecies.

Table 1 CSF D-lactate and L-lactate levels and four other CSF inflammatory parameters in different forms of meningitis

Diagnosis	Leukocytes M/l[1]	Erythrocytes M/l[1]	Total Protein g/l[1]	L-Lactate mmol/l[1]	D-Lactate mmol/l[1]	D-Glucose mg/dl[1]
Controls[a] lumbar (n=50)	1 0-5	2 0-190	0.36 0.23-0.51	1.39 0.97-2.43	0.02 0.00-0.15	58 49-82
Controls[a] cisternal (n=8)	1 1-3	5 0-20	0.27 0.16-0.33	1.33 0.93-1.63	0.00 0.00-0.10	55 49-60
Bacterial Meningitis[b] acute (n=12)	447 168-21,504	620 35-20,000	1.90 0.70-6.58	7.57 3.91-17.85	0.47 0.28-1.28	52 34-72
Bacterial Meningitis[c] acute (n=11)	13 2-128	320 0-20,000	0.79 0.40-5.00	2.90 1.50-5.19	0.08 0.00-0.18	49 15-99
Bacterial Meningitis[d] treated (n=18)	9 3-46,080	419 0-10,000	0.63 0.15-5.65	4.76 2.16-13.00	0.06 0.00-0.43	66 23-157
Meningitis[e] (n=89) untreated and treated	40 0-9,216	2,125 0-999,999	0.90 0.21-13.88	3.20 0.91-7.14	0.02 0.00-0.18	73 25-120

[1] Median values with 1. and 99. percentiles. D- and L-lactate were measured enzymatically [9,11] using manual and mechanized UV tests from Boehringer Mannheim. Other constituents were determined as described [2-4].
[a] patients, 13 to 77 years old, without inflammatory and severe neurological symptoms.
[b] CSF mainly from ventricular drainage containing *S. pneumoniae, Staphylococcus aureus* and other unclassified coccae.
[c] CSF mainly from ventricular drainage containing unclassified coccae and bacteria.
[d] CSF mainly from ventricular drainage containing sporadically few bacteria.
[e] Lumbar, cisternal and ventricula CSF samples which were negative with Particle Counting Immunoassays (PACIA) [7] for *H. influenzae* type B, *S. pneumoniae*, N. *meningitidis* types A, B, C, *Streptococcus group* B, *Staphylococcus* spp. and candida spp. as well.

(table 1). However, in 11 samples of ventricular CSF samples containing bacteria, D-lactate content was < 0.20 mmol/l (table 1). Since other parameters of acute bacterial meningitis, e.g. leukocyte counts, L-lactate, were mostly negative (table 1) our data may indicate a bacterial contamination of the ventricular drainage. Moreover, our data point to some bacteria which are unable to produce D-lactate *in vivo* as it was shown *in vitro* [8].

We used the same cut-off value of ≥ 0.20 mmol/l D-lactate in NaF-plasma to diagnose a systemic bacterial inflammation in blood [10]. Since D-lactate levels in blood influence those in CSF [10], D-lactate levels in CSF ≥ 0.2 mmol/l only indicate a bacterial inflammation when D-lactate levels in plasma are lower than those in CSF [10]; D-lactate levels of ≥ 0.2 mmol/l in blood plasma indicate an extracerebral infection with D-lactate producing bacteria [10].

In summary, L-lactate levels in CSF ≥ 3.5 mmol/l point with high certainty to a bacterial meningitis when leukocyte counts were ≥ 800 M/l. With leukocyte counts < 800 M/l a positive detection of bacterial antigens or lysozyme levels ≥ 3 mg/l together with L-lactate levels ≥ 3.5 mmol/l indicate a bacterial meningitis even in treated cases with sterile cultures. D-lactate proves to be useful in specific diagnosis of meningitis with D-lactate producing bacteria when CSF levels exceed those in blood plasma.

Acknowledgement

This work was supported by the Bundesminister für Bildung, Wissenschaft, Forschung und Technologie (BMFT) with MEDWIS-Vorhaben Az: 103/1.

References

1. Kleine TO, Baerlocher K, Niederer V, Keller H, Reutter F, Tritschler W, Bablok W. Diagnostische Bedeutung der Lactatbestimmung im Liquor bei Meningitis. Dtsch Med Wschr 1979;104:553-7.
2. Kleine TO, editor. Neue Labormethoden für die Liquordiagnostik. Stuttgart(Deutschland): Thieme, 1980.
3. Kleine TO. Diagnostische Untersuchungen im Liquor cerebrospinalis. In: Thomas L. editor. Labor und Diagnose. Marburg (Deutschland): Medizinische Verlagsgesellschaft. 1984:937-65.
4. Kleine TO. Nervensystem. In: Greiling H, Gressner AM, editors. Lehrbuch der Klinischen Chemie und Pathobiochemie. 1. Auflage 1987. 2. Auflage 1989. Stuttgart, New York: Schattauer 1987,1989:860-93.
5. Kleine TO. Evaluation of three polymorphonuclear leukozyte

constituents in CSF and comparison with lactate content and leukocyte counts for the discrimination of meningitis. J Clin Chem Clin Biochem 1989;27:926-8.
6. Kleine TO. Valuation of bacterial and viral antigen detection against lactate determination and leukocyte counts in CSF meningitis diagnosis. J Clin Chem Clin Biochem 1989;27:901-3.
7. Kleine TO, Cambiaso CL. Grenzen des manuellen und voll-mechanisierten Nachweises von Erreger-spezifischen Antigenen im Liquor cerebrospinalis. Lab med 1991;15:109-13.
8. Janke KH, Kleine TO, Nebel P, Mannheim W. D-lactate and L-lactate production by bacteria causing meningitis and sepsis. J Lab Med 1996;20: 508-10.
9. Kleine TO. D-Lactat und L-Lactat im Liquor cerebrospinalis bei akuten entzündlichen Erkrankungen des Zentralnervensystems. Lab med 1991;15:114-6.
10. Kleine TO, van der Meij RA, Dauch WA, Lütcke A. Lactatdifferenzierung im Liquor und Blutplasma bei der Akut-Diagnostik zerebraler Notfälle. Intensiv- und Notfallbehandlung 1992:17:134-7.
11. Kleine TO, Nebel P, Naumann-Haak C, Diergardt P. Vollenzymatische Messung von D- und L-Lactat im Liquor cerebrospinalis. In: Günther W, Hempel V, Wulff G, editors. InCom'94. International Symposium on Instrumentalized Analytical Chemistry and Computer Technology. Düsseldorf(Deutschland): InCom-Bureau IAS 1994:285.

Topic IV: Poster Abstracts*

Häufigkeit entzündlicher Liquorveränderungen bei Neuritis nervi optici

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Die Diagnostik einer Neuritis nervi optici (NNO) stützt sich auf den ophthalmologischen und neurologischen Befund, das zerebrale MRT (einschließlich Beurteilung des N. opticus) sowie den Liquorbefund, dessen prognostische Bedeutung hinsichtlich einer Multiplen Sklerose (MS) kontrovers diskutiert wird [1]; die Entwicklung einer MS wird in der Literatur zwischen 13 und 88% angegeben [2]. - 10 Patienten (Alter: 14-43 Jahre) mit klinisch-ophthalmologisch gesicherter NNO wurden neurologisch, kernspintomographisch und liquorologisch untersucht. Im zerebralen MRT zeigten 3/10 Patienten multiple demyelinisierende Herdbildungen. Der lumbale Liquor erbrachte eine geringe Zellzahlerhöhung (5-27 Mpt/l) bei 6/10 Fällen, Plasmazellen wurden bei 8/10 nachgewiesen. Eine leichte Schrankenfunktionsstörung ergab sich bei 1/10. Aktivierte B-Lymphozyten zeigten sich bei 9/10 (IgG), 5/10 (IgA) und 4/10 (IgM). Eine intrathekale IgG-Synthese (Schema n. Reiber) wurde bei 5/10 (IgG), 2/10 (IgA) und 2/10 (IgM) festgestellt. Die Bestimmung von oligoklonalem IgG erwies sich bei 9/10 Patienten als positiv. Hinweise auf eine polyspezifische Antikörpersynthese im zentralen Nervensystem (MRZ-Reaktion) waren bei 7/10 vorhanden (MRZ positiv 1/7, RZ positiv 3/7, R positiv 1/7, M positiv 2/7). Langzeitbeobachtungen bei NNO und entzündlichen Liquorveränderungen, wie oligoklonale Banden, und positive MRZ-Reaktion könnten Hinweise auf das Risiko der Entwicklung einer MS ergeben.

1. Söderström M, Linqvist M, Hillert J, Kall TB, Link H. Optic neuritis:finding on MRI, CSF examination, HLA class II typing in 60 patients and results of short-term follow-up. J Neurol 1994;241:391-7.
2. Kurtzke JF. Optic neuritis or multipe sclerosis. Arch Neurol 1985;42:704-10.

Determination of Avidity Distribution of Antibodies: Methodological Aspects

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Low avidity antibodies may indicate that the affinity maturation is impaired or that the antibodies are primarily directed against a similar antigen to which they have high avidity. Therefore, the detection of low-avidity antibodies should have a diagnostic value. Chaotropic ions (e.g. SCN-) interact with water causing increased freedom for water molecules, i.e. the entropy of water is increased. This affects the dissolving properties of water causing low avidity antigen-antibody complexes to dissolve. *Methods.* The avidity spectrum is determined by an ELISA technique primalily determining the amount of specific antibodies. Subsequently different concentrations of NaSCN are added, splitting low avidity antibody-antigen bindings. *Principles for improvement.* At present the technique has only been used in research. The method is not yet ready for a routine use. There main drawbacks are (a) The method is laborious, (b) The concentration of NaSCN is varied in steps and not continuosly, (c) The interpretation of the result should be improved, (d) The clinical value is not yet established, (e) Reliable performance data (precision, trueness etc.) are still lacking.

Reference. Vrethem M, Ekerfelt C, Ernerudh J. Avidity distribution of antibodies against nerve myelin in patients with polyneuropathy associated with IgM monoclonal gammopathy and in healthy controls. J Neurol Sci 1995;131:190-9.

Evaluation of the Intrathecal Specific Immune Response in Neuroborreliosis by Immunoblot

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Objective: To investigate whether the different manifestations and stages of neuroborreliosis are characterized by individual patterns of their immunological *Borrelia burgdorferi* (Bb) specific central nervous

* Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic IV, Marburg an der Lahn, September 29 and 30, 1995

system (CNS) response. *Background:* Bb can cause a broad variety of peripheral and CNS complications of differing severity and prognosis, which might be due to different immune mediated pathomechanisms. *Methods:* The IgG and IgM response against 12 antigens of Bb was analysed by the immunoblot technique in 90 untreated patients with a serological proven neuroborreliosis stage II (n = 82) and III (n = 8). A local antigen specific immune response was assumed if an antibody band in the cerebrospinal fluid (CSF) was stronger than in the serum diluted to the same IgG content as CSF. *Results:* In stage II the number of intrathecally produced antibody bands was in average 1.4 (IgM) and 2.8 (IgG). Comparing groups of different disease duration a limited expansion of the immune response was observed (O-1 month: 2.6 IgG bands; 1-6 months: 3.1 IgG bands) . The antibody response was mainly directed against the p41-(flagellin) (81.7%) and p66- (31.7%) antigen. High specific, surface located antigens were recognized in a lower frequency (e.g. p21: 18.3%). In 28% of the patients CSF restricted antibodies could be depicted. In contrast patients of stage III had a more heterogenous (IgM bands: 3.1. IgG bands: 7.0) and intensive immune response directed mainly against specific antigens (e.g. p21: 62.5%). In the mayority of patients CSF restricted antibodies could be found (87.5%). Comparing different clinical features in stage II (meningitis, meningoradiculitis, meningomyelitis, meningoencephalitis) no differences were found regarding heterogeneity and specificity of the intrathecal immune response. *Conclusion:* The heterogeneity and specificity of the intrathecal immune response differs between stages but not in clinical features of stage II neuroborreliosis corresponding to the known self limited or chronic progressive course of the disease. The highly specific antibody pattern in stage III supports the theory of an ongoing infection rather than an autoimmune process. Therefore the broadening of the antigen specificity of the intrathecal immune response over the time course might be a marker for a persistent infection.

Frequency of Virus Specific Antibodies in Clinically Definite Multiple Sclerosis versus Acute Monosymptomatic Opticus Neuritis

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Isolated optic neuritis (ON) is a common first manifestation of multiple sclerosis (MS). To investigate whether the polyspecific immune response against neurotropic viruses (measles, rubella, varicella zoster, mumps), described for 94% of patients with definite MS, is also already present in the early phase of the disease, a group of 67 patients (30.3 years, 49 women) with ON was compared to 50 MS patients (41.9 years, 32 women). Increased antibody specificity indices (ASI > 1.4) against at least 1 of the 4 viruses were present in 43 (86%) of the MS patients and in 41 (61.2%) of the ON cases. Whereas a single antigen response occurred in only 11/43 (26.6%) of MS patients, it dominated in 17/41 (41.5%) of the ON patients. Measles was the antigen most frequently recognized in MS (32/43; 65.3%) and ON (25/64; 39,1%), followed by rubella (56.6% / 32.3%), zoster (51.0% / 33.8%) and mumps (33.3% / 23.2%). The intensity of a particular ASI (except mumps) correlated with the amount of intrathecally synthesized IgG in both groups. Although the antiviral immune reaction in ON generally resembled the one in MS, it was weaker in frequency and intensity. Nevertheless 6 out of 17 ON patients, who lacked CSF oligoclonal bands, exhibited slightly raised single ASI values bringing the number of ON patients with any immunological abnormality to a total of 56/67 (83.6%), thus adding evidence to the notion that ON represents a form fruste of MS.

Cerebrospinal Fluid Apolipoproteins and Blood/Cerebrospinal Fluid Barrier*

Liquor Apolipoproteine und Blut/Liquor Schranke*

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Keywords: Acquired Immunodeficiency Syndrome/cerebrospinal fluid; Apolipoproteins/cerebrospinal fluid, Serum Amyloid A/cerebrospinal fluid; Immunoassay; Multiple Sclerosis/cerebrospinal fluid; Cerebrospinal Fluid.

Schlüsselwörter: AIDS/Liquor cerebrospinalis; Apolipoproteine/Liquor cerebrospinalis; Serum Amyloid A/Liquor cerebrospinalis; Immunoassay; Multiple Sklerose/Liquor cerebrospinalis; Liquor cerebrospinalis.

Twelve specific lipid binding proteins, named apolipoproteins (apos), are identified presently in the normal cerebrospinal fluid (CSF). We investigated 7 of them in a population of 74 patients: 29 definite multiple sclerosis (MS), 21 AIDS (state IV) and 24 other neurological diseases patients (OND) compared to 10 subjects without neurological symptoms (controls).

The blood/CSF ratio of *apo A-I* (table 1) was very high in the control group. In various diseases an increase in CSF A-I was observed and an intrathecal synthesis of apo A-I cannot be excluded, but not be asserted today.

The determination of CSF *apo A-II* was performed using a sensitive ELISA, with a monoclonal antibody: We previously demonstrated the considerable decrease in apo A-II in the serum of AIDS patients [1], contrasting with its increase in the CSF, i.e.: its very probable intrathecal synthesis in this disease.

The very low concentrations of *apo B* and *apo (a)* in CSF need a very sensitive immunofluorometric method [2,3] to be measured. The blood/CSF ratio of each apo was extremely high (7000 and 4000, respectively) suggesting that an intrathecal synthesis of both these apos is very improbable.

Table 1 Apolipoprotein A I (means ± S.E.) in serum and cerebrospinal fluid (CSF)

	Serum mg/l	Cerebrospinal Fluid mg/l
Controls (n=10)	1551 ± 214	4.9 ± 0.8
M.S.[1] (n=29)	1593 ± 87	8.5 ± 0.9
AIDS[2] (n=21)	1310 ± 70	8.7 ± 0.7
OND[3] (n=24)	1352 ± 85	8.8 ± 0.9

[1] multiple sclerosis (MS); [2] aquired immune deficiency syndrome (AIDS, state IV); [3] other neurological diseases patients (OND).
Statistical evaluation: in serum: MS versus OND: $p < 0.04$; in CSF: controls versus MS: $p < 0.02$, versus OND: $p < 0.02$, versus AIDS: $p < 0.01$.

Apo H (previously called β_2-glycoprotein 1) seems of minor interest, and no intrathecal synthesis may be suspected.

Serum amyloid A is an acute phase protein. Using a monoclonal ELISA we established its presence in the serum and the CSF of the control group. Its intrathecal synthesis seems very common in AIDS patients and in some other inflammatory processes.

More interesting is *apo E,* which is involved in peripheral myelin repair [4] and synthesized by astrocytes in culture. Its determination was performed [5] by a time-resolved immunofluorometric assay using a polyclonal antibody (a generous gift of Professor *Baudner,* Behring Institut, Marburg) which was labelled with europium. Its very low blood/CSF ratio of 12 in the control group suggests an intrathecal synthesis. This was calculated from the theoretical blood/CSF ratio (expected for a 34 kDa protein) which was evaluated to 110. In cases where CSF albumin is higher than its normal mean (210 mg/l) this ratio is multiplied by the ratio CSF albumin/210. In our controls (table 2) a daily intrathecal synthesis was evaluated to 2.5 mg: 80 to 90% of CSF apo E derives from this intrathecal synthesis. In the multiple sclerosis group a slight increase

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* Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic V, Marburg an der Lahn, September 29 and 30, 1995
Nonstandard abbreviations: AIDS, aquired immune deficiency syndrome; apo, apolipoprotein; CSF, cerebrospinal fluid; ITS, intrathecal synthesis; kDa, kilodalton; MS, multiple sclerosis; OND, other neurological diseases patients; S.E., standard error.

Table 2 Apolipoprotein E in serum and cerebrospinal fluid (CSF) and its intrathecal synthesis rate (ITS) (means ± S.E.)

	Serum mg/l	CSF mg/l	ITS mg/l	ITS/CSF
Controls (n=10)	66.7 ± 3.8	5.5 ± 0.3	4.8 ± 0.3	0.87
M.S.[1] (n=29)	80.1 ± 3.9	3.3 ± 0.4	2.4 ± 0.4	0.73
AIDS[2] (n=21)	74.5 ± 5.5	5.7 ± 0.5	4.7 ± 0.4	0.82
OND[3] (n=24)	60.7 ± 3.7	4.7 ± 0.5	3.8 ± 0.5	0.81

[1] Multiple sclerosis (MS); [2] aquired immune deficiency syndrome (AIDS, state IV); [3] other neurological diseases patients OND).
Statistical evaluation [ ]: in serum: MS versus OND: $p < 0.01$; in CSF: MS versus controls: $p < 0.01$, versus AIDS: $p < 0.01$, versus OND: $p < 0.01$; in ITS: MS versus controls $p < 0.001$, versus AIDS: $p < 0.001$.

in serum apo E contrasts with a significant decrease in CSF apo E, i.e.: of its intrathecal synthesis rate. Such decrease seems significantly linked to the duration of multiple sclerosis: Intrathecal synthesis mean is significantly lower after 10 years from the onset of the disease. These new data suggest a defect in lipid turnover, which may slow (or even block) myelin repair. Such defect seems restricted to the glial cells which produced apo E.

References

1. Gervais A, Ayrault-Jarrier M, Reboul J, Schuller E, Meillet D, Apartis E, et al. Apolipoproteins A II in HIV-1 infection. Lancet 1992;ii 340:730-1.
2. Osman I, Gaillard O, Meillet D, Bordas-Fonfrede M, Gervais A, Schuller E, et al. A sensitive time-resolved immunofluorometric assay for the measurement of apolipoprotein B in cerebrospinal fluid. Application to multiple sclerosis. Eur J Clin Chem Clin Biochem 1995;33:53-8.
3. Gaillard O, Meillet D, Gervais A, Delattre J, Galli J, Schuller E. Lipoprotein (a) in cerebrospinal fluid measured by highly sensitive time-resolved immunofluorometric assay. Clin Chem (Letter) 1994;40:1975-6.
4. Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, Weisgraber KH, et al. A role for apolipoprotein E, apolipoprotein A I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. J Clin Invest 1989;83:1015-31.
5. Gaillard O, Gervais A, Meillet D., Delattre J, Lyon-Caen O, Schuller E. Apolipoprotein E intrathecal synthesis is decreased in multiple sclerosis patients. Ann Clin Biochem 1996;33:148-50.

Activation of the Classical or the Alternative Pathway of the Complement System in Cerebrospinal Fluid during Various Diseases of the Central Nervous System*

Aktivierung des klassischen oder alternativen Weges im Komplement System im Liquor cerebrospinalis während verschiedener Erkrankungen des Zentralnervensystems*

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Keywords: Alzheimer's Disease; Cerebrospinal Fluid; Complement Activation; Cerebrospinal fluid; Membrane Attack Complex/cerebrospinal fluid; Lupus Erythematosus, Systemic; Meningitis, Bacterial; Meningitis, Viral; Multiple Sclerosis; Polyradiculoneuritis.

Schlüsselwörter: Alzheimer Krankheit; Liquor cerebrospinalis; Komplementaktivierung; C3a-desArg, C5a-desArg/Liquor cerebrospinalis; Membranangriffskomplex/Liquor cerebrospinalis; Lupus erythematodes, systemischer; Meningitis, bakterielle; Meningitis, virale; Multiple Sklerose; Polyradiculoneuritis.

In many neurological diseases tissue damage is mediated, at least in part, by components of the immune system. Both cellular and humoral elements have been implicated in neurological diseases. So far evidence of complement playing a distinct role, has been obtained only in a small number of diseases.

Serum from patients with *Guillain-Barré syndrome* contains an antibody against Schwann cells, which in the presence of complement, causes demyelination of myelinated nerve cultures *in vitro*. Elevated levels of complement activation products C3a-desArg, C5a-desArg, C4d and the terminal complement complex TCC have been found in cerebrospinal fluid (CSF). This provides evidence of complement activation in the central nervous system (CNS), although the relevance to the pathogenesis for a disease that primarily affects the peripheral nerve system is unclear [1-3].

Results supporting evidence for the activation of the complement system in patients with *multiple sclerosis* have been contradictory. For example CSF levels of C3 or C4 have been found to be elevated, normal or lower [4-6]. Since these proteins are not valid indicators of activation, activation products were measured instead. Compared with the control group, where in most of the cases the activation products were below the detection limit (in 88 to 93%), we were able to detect complement activation in 73% of these patients measuring C3a-desArg. The alternative convertase (= activation via the alternative pathway) was elevated in 43% and the C1rsC1 Inactivator (= activation via the classical pathway) was elevated in 27% of the cases. The range of C3a-desArg concentration was 5-90 ng/ml CSF. Most of the patients had levels about 20-30 ng/ml. Low levels of C9 were found and TCC was detectable, suggesting terminal complement activation in the CNS [7, 8]. Using C8-depleted serum in an *in vitro* model system, demyelination did not occur implicating the action of the terminal complement complex.

In *Alzheimer's disease* C3, C4 and C1 have been identified in senile plaques and decreased levels of C1q were detected in CSF [9]. In patients with *systemic lupus erythematosus* with CNS involvement, a pathogenic role for complement has been proposed finding decreased levels of plasma C4, an increase of CSF C4 index and an increase in TCC levels in the CSF [10, 11].

In the initial stage of *subarachnoid hemorrhage* an increase of C3a-desArg and C5a-desArg was found in CSF. Activation of the complement system may have an effect on vascular tone and may thus play a central role in the development of cerebral vasospasm. It has been shown that complement activation coincided with the generally accepted time schedule for development of cerebral vasospasm [12]. However, it has not been clarified whether or not the activation is causative for the development or if it represents an epiphenomenon or a non-specific reaction to cerebral injuries.

In CSF of patients with *bacterial* or *viral meningitis* we found elevated concentration of C3a-desArg in all patients compared to a control group of patients with

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* Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic V, Marburg an der Lahn, September 29 and 30, 1995

Nonstandard abbreviations: CSF, cerebrospinal fluid; CNS, central nervous system; TCC, terminal complement complex.

non-inflammatory diseases of the CNS. The C3a-desArg concentration in CSF of the control group was below the detection limit of 1-2 ng/ml CSF in 88% of the patients. There was also a significant increase of C3a-desArg in viral meningitis compared to the control group.

This increase in C3a-desArg was mainly due to alternative pathway activation. In bacterial and in viral meningitis a significant increase in alternative convertase C3bBbP was found compared to the control group. C3bBbP concentrations were found to be below the detection limit in 84% of controls. There was a significant increase in concentration of total protein, IgG and lactate in CSF of patients with bacterial meningitis. However, no correlation between C3a-desArg and total protein or IgG in CSF of meningitis patients was found. There was, however, a good correlation between C3a-desArg and lactate in CSF in case of bacterial and viral meningitis.

Plasma levels of C3a-desArg were significantly elevated in patients with bacterial meningitis due to systemic activation of the complement system. However, there was no correlation between C3a-desArg in plasma and CSF. In some patients with extremely high C3a-desArg concentration in plasma, C3a-desArg in CSF was only slightly elevated. On the other hand high concentration of C3a-desArg in CSF could be found in cases of relatively low concentration of plasma C3a-desArg. Therefore, increase of the activation products in CSF seems to reflect an intrathecal complement activation.

Whether determination of CSF complement activation products can prove to be useful in monitoring disease activity and effectiveness of treatment regiments still needs to be proven.

References

1. Hartung HP, Schwenke C, Bitter-Suermann D, Toyka KV. Guillain-Barre syndrome: Activated complement components C3a and C5a in CSF. Neurology 1987;37:1006-9.
2. Koguchi Y, Yamada T, Kuwabara S, Nakajima M, Hirayama K. Increased CSF C4d in demyelinating neuropathy indicates the radicular involvement. Acta Neurol Scand 1995;91:58-61.
3. Liu WT, Vanguri P, Shin ML. Studies on demyelination in vitro: the requirement of membrane attack components of the complement system. J Immunol 1983;131:778-82.
4. Jans H, Heltberg A, Zeeberg I, Kristensen JH, Fog T, Raun NE. Immune complexes and the complement factors C4 and C3 in cerebrospinal fluid and serum from patients with chronic progressive multiple sclerosis. Acta Neurol Scand 1984;69:34-8.
5. Yam P, Petz LD, Tourtelotte WW, Ma BI. Measurement of complement components in cerebral spinal fluid by radioimmunoassay in patients with multiple sclerosis. Clin Immunol Immunopath 1980;17:492-505.
6. Price P, Cuzner ML. Cerebrospinal fluid complement proteins in neurological disease. J Neurol Sci 1980;46:49-54.
7. Morgan BP, Campbell AK, Compston DAS. Terminal component of complement (C9) in cerebrospinal fluid of patients with multiple sclerosis. Lancet 1984:251-4.
8. Mollnes JE, Vandvik B, Lea T, Vartdal F. Intrathecal complement activation in neurological diseases evaluated by analysis of the terminal complement complex. J Neurol Sci 1987;78:17-28.
9. Smyth MD, Cribbs DH, Tenner AJ, Shankle WR, Dick M, Keslak JP, et al. Decreased levels of C1q in cerebrospinal fluid of living Alzheimer patients correlate with disease state. Neurobil Aging 1994;15:609-14.
10. Jongen PJ, Boerbooms AM, Lamers KJ, Raes BC, Vierwinden G. Diffuse CNS involvement in systemic lupus erythematosus: intrathecal synthesis of the 4th component of complement. Neurology 1990;40:1593-6.
11. Sanders ME, Alexander EL, Koski CL, Frank MM, Joiner KA. Detection of activated complement (C5b-9) in cerebrospinal fluid from patients with central nervous system involvement of primary sjogrens syndrome or systemic lupus erythematosus. J Immunol 1987;138:2095-9.
12. Kasuya H, Shimizu T. Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. J Neurosurg 1989;71:741-6.

Proteolytic Activity in Cerebrospinal Fluid and Demyelination*

Proteolytische Aktivität im Liquor cerebrospinalis und Demyelinisierung*

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Keywords: Acquired Immundeficiency Syndrome; Cerebrospinal Fluid; Demyelinating Diseases; Electrophoresis, Polyacrylamide Gel; Encephalitogenic Basic Proteins/metabolism; Viruses; Proteinases/analysis; Serine Proteinases/cerebrospinal fluid.

Schlüsselwörter: AIDS; Elektrophorese, Polyacrylamidgel; Encephalitis-verursachendes basisches Protein/Stoffwechsel; Entmarkungskrankheit; Liquor cerebrospinalis; Viren; Proteinasen/Analytik; Serinproteinasen/Liquor cerebrospinalis.

Proteolytic enzymes are possible mediators of demyelination and their activity increases in inflammatory demyelinating diseases such as multiple sclerosis.

We are studying the involvement of proteases in myelin breakdown by three different approaches.

The *first approach* is to assess the presence of proteolytic activity in the cerebrospinal fluid (CSF) by SDS gel electrophoresis after incubation of CSF samples with purified myelin basic protein (MBP). This is a representative substrate to study myelin degradation and its presence in CSF is a direct marker of myelin breakdown [1].

In our study on AIDS patients with neurological complications, we have found that anti-MBP CSF proteolytic activity increases and seems to have a role only in neurological complications of a viral nature such as progressive multifocal leukoencephalitis and AIDS dementia complex, in which demyelination is one of the main pathological alterations, but not in other opportunistic non-viral infections in which demyelination is only a transient event. The anti-MBP proteolytic activity was inhibited by serine protease inhibitors and therefore is not associated to myelin [2].

The *second approach* is to identify CSF proteases that are specific for the target. To this end we use zymography, an electrophoretic technique on gel copolymerized with the substrate showing proteases as white bands on a blue background. We used two substrates: gelatin, commonly used for these purposes (but not a myelin component), and MBP, as a specific myelin component. Different patterns were obtained. Up to 7 bands with apparent MWs between 63 and 132 kDa were observed on gelatin, whereas four bands of 87, 83, 79 and 72 kDa were observed on MBP-containing gels. Among the latter, the 87 and 79 kDa bands were more specifically related with myelin breakdown, as assessed by CSF-MBP level and neuroimaging findings, and were not present on gelatin gels.

The *third approach* is to set up methods to recognize the origin of the proteases acting on myelin and the regulation of their production: cell type, sensitivity to cytokines and inhibitors. To start with this point we have chosen a well defined experimental disease in which demyelination is induced in the mouse by a virus, the Theiler's murine encephalomyelitis virus (TMEV). In our study we have compared TMEV-infected macrophages and microglia, isolated from both susceptible SJL/J and resistant C57BL/6 mice, for their ability to secrete proteolytic enzymes capable of degrading MBP. As detected by SDS gel electrophoresis, the activity of a serine protease was found only in supernatants from infected SJL/J, but not from C57BL/6, microglia and macrophages [3].

In conclusion, our results suggest a role for proteolytic enzymes, and in particular of serine proteases in virus-induced demyelination. The use of an original zymographic method on MBP-gels is now leading to the identification of these enzymes. This is the prerequisite for the identification of their origin and for the understanding of their mechanism of action on myelin.

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* Presented at the European CSF-Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic V, Marburg an der Lahn, September 29 and 30, 1995

Nonstandard abbreviations: CSF, cerebrospinal fluid; kDa, kilodalton; MBP, myelin basic protein; SDS, sodium dodecyl sulfate; TMEV, Theiler's murine encephalomyelitis virus.

Acknowledement

Supported by Grant TSS 9306-12, AIDS Pr.9S, Italy.

References

1. Liuzzi GM, Mastroianni CM, Vullo V, Jirillo E, Delia S, Riccio P. Cerebrospinal fluid myelin basic protein as predictive marker of demyelination in AIDS dementia complex. J Neuroimmunol 1992;36:251-4.
2. Liuzzi GM, Mastroianni CM, Fanelli M, Massetti AP, Vullo V, Delia S, Riccio P. Myelin degrading activity in the CSF of HIV-1-infected patients with neurological diseases. Neuroreport 1994;6:157-60.
3. Liuzzi GM, Riccio P, Dal Canto MC. Release of myelin basic protein-degrading proteolytic activity from microglia and macrophages after infection with Theiler's murine encephalomyelitis virus: Comparison between susceptible and resistant mice. J Neuroimmunol 1995;62:91-102.

Approach to Measure Native Proteolytic Activity in Human Cerebrospinal Fluid with a Fluorophore Substrate*

Zur Messung von nativer proteolytischer Aktivität im menschlichen Liquor cerebrospinalis mit einem fluorophoren Substrat*

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Keywords: Blood-Brain Barrier; Calpain/cerebrospinal fluid; Calpain/antagonists and inhibitors; Central Nervous System; Cerebrospinal Fluid. Inflammation; Peptide Hydrolases/cerebrospinal fluid.

Schlüsselwörter: Blut-Hirn-Schranke; Calpain/Liquor cerebrospinalis; Calpain/Antagonisten und Inhibitoren; Entzündung; Liquor cerebrospinalis; Peptidhydrolasen/Liquor cerebrospinalis; Zentralnervensystem.

Different proteases are involved in inflammatory processes with demyelination in the central nervous system (CNS), e.g. induced by virus infection [1] or during multiple sclerosis with lesion formation and enlargement [2]. Proteolytic enzyme activity was detected in cerebrospinal fluid (CSF) [3-5], but its origin is unclear: It can be released from microglia [6] and neurons [7] as well as from macrophages [8] or granulocytes [3].

Since few data are known how to measure exo- and endo-proteases in CSF, e.g. serine proteases, cysteine proteases, aspartate proteases, metalloproteases, we established a simple routine assay to determine protease activity in CSF under physiological conditions. As substrate we used bovine milk casein coupled with six resorufin molecules per molecule [9]. We found native proteolytic activity in lumbar CSF under normal and pathologic conditions (fig. 1).

Since cell surface peptidases expressed on leukocytes, e.g. enkephalinase (EC 3.4.24.11), aminopeptidases A, N (EC 3.4.11.7, EC 3.4.11.2), and other membrane bound peptidases, e.g. angiotensin I converting enzyme (EC 3.4.15.1) in human brain, only cleave peptides [7], other proteases come into question which cleave casein, resorufin-labelled: Ca^{2+} activated neutral protease (EC 3.4.22.17: calpain) from human brain [10] besides other high-molecular weight proteases e.g. from blood plasma. From the intracellularly located proteases, cathepsins (B.H.L) are only active at acid pH.

We tried to classify low levels of proteolytic activity in lumbar CSF by specific activators and inhibitors [cf. 11]: *Native proteolytic* activity (fig. 1A) contained some calpain activity (mostly inhibited by calpastatin I [10]), few elastatinal inhibited activity (e.g. elastase found in microglia [12]) besides other proteolytic activity at pH 7.4 which may derive from blood plasma. It was somewhat higher in CSF from patients with CNS inflammation, but considerably lower in blood plasma.

Ca^{2+} activated proteolytic activity *(calpain)* (fig. 1B) was mostly inhibited by calpastatin II [10]: it was somewhat higher with bbb disturbances and CNS inflammations (fig. 1B). It may derive from brain cells because it did not correlate with leukocyte counts in CSF and its activity was considerably lower in blood plasma. Highest activity (100 µmol/l/24h) was determined in CSF from acute bacterial meningitis.

In summary, native proteolytic activity in human CSF determined with casein, resorufin-labeled, at pH 7.4, consists of different neutral proteases e.g. calpain, elastase, which may not stem from blood plasma. Different cleavage properties of the proteases against casein [9] have to be considered when performing the new assay in CSF and blood plasma. Since native proteolytic activity strongly increased in CSF with acute bacterial meningitis it may play an important rolé in controlling of inflammatory processes in CNS.

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* Presented at the European CSF Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic V, Marburg an der Lahn, Germany, September 29 and 30, 1995

Nonstandard abbreviations: bbb, blood-brain-barrier; CNS, central nervous system; CSF, cerebrospinal fluid.

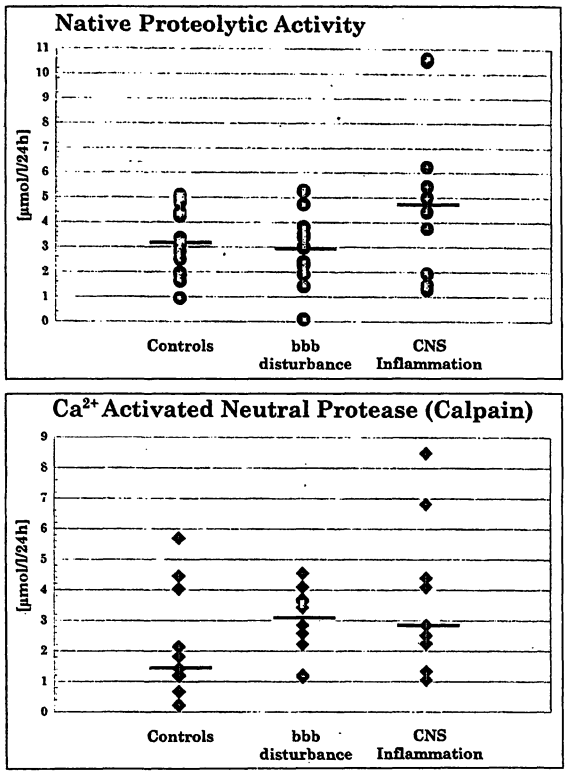

Figure 1 Demonstration of protease activity (µmol/l CSF/24 h) in lumbar CSF samples from *controls* (patients (n=15) undergoing a lumbar myelography and exhibiting normal cell and protein values in CSF); from patients (n=14) showing *bbb disturbance* without a pleocytosis; from patients (n=10) exhibiting different *CNS inflammation* with pleocytosis. Median values are indicated as a line from native protease activity (fig. 1A) and calpain (fig. 1B). Proteolytic activity was determined at 37 °C and pH 7.4 in a total volume of 0.100 ml consisting of 0.050 ml CSF, casein, resorufin-labelled (Boehringer Mannheim), Tris buffer, sodium azide, activators and inhibitors [cf. 11]. Colour released from the fluorophore substrate was measured at 578 nm in the supernatant after precipitation with trichloroacetic acid [9].

Acknowledgement

This work was supported by the Bundesminister für Bildung, Wissenschaft, Forschung und Technologie (BMFT) with MEDWIS-Vorhaben Az: 103/1.

References

1. Liuzzi GM, Mastroianni CM, Fanelli M, Massetti AP, Vullo V, Delia S, Riccio P. Meylin degrading activity in the CSF of HIV-1-infected patients with neurological diseases. Neuroreport 1994;6:157-60.
2. Bever CT, Whitaker JN. Proteinases in inflammatory demyelinating diseases. Springer Semin Immunopathol 1985;8:235-50.
3. Cuzner ML, Davison AN, Rudge P. Proteolytic enzyme activity of blood leukocytes and cerebrospinal fluid in multiple sclerosis. Ann Neurol 1978;4:337-44.
4. Inuzuka T, Sato S, Baba H, Miyatake T. Neutral proteases in cerebrospinal fluid of patients with multiple sclerosis and other demyelinating diseases. Acta Neurol Scand 1987;76:18-23.
5. Riccio P, Liuzzi GM. Proteolytic activity in cerebrospinal fluid and demyelination. J Lab Med 1996;20:520-1.
6. Nakajima K, Kohsaka S. Functional roles of microglia in the brain. Neurosci Res 1993;17:187-203.
7. Turner AJ, editor. Neuropeptides and their peptidases. Ellis Horwood Series in Biomedicine. Weinheim (Deutschland): VCH, 1987.
8. Einstein ER, Csejtey J, Dala KB, Adams CWM, Bayliss OB, Hallpike JF. Proteolytic activity and basic protein loss in and around multiple sclerosis plaques: combined biochemical and histochemical observations. J Neurochem 1972;19:653-62.
9. Kleine TO, Lenz W, Mülheim J, Peterson W. Measuring of protease activity by photometric and radiometric methods in skeletal muscle: a comparison. Eur J Clin Chem Clin Bioch 1995;33:A33-4.
10. Murachi T, Hatanaka M, Hamakubo T. Calpains and neuropeptide metabolism. In: Turner AJ, editor. Neuropeptides and their peptidases. Weinheim (Deutschland): VCH, 1987:202-28.
11. Kleine TO, Lenz W. Model effect of tonic stretch in chicken wing muscles: induction of acid and neutral proteinase activity. In: Wegmann RJ, Wegmann MA, editors. Recent Advances in Cellular and Molecular Biology. Leuven(The Netherlands): Peeters Press, 1992;5:57-68.
12. Nakajima K, Shimojo M, Hamanoue M, Ishiura S, Sugita H, Kohsaka S. Identification of elastase as a secretory protease from cultured rat microglia. J Neurochem 1992;58:1401-8.

Topic V: Poster Abstracts*

Tetranectin (TN) in Cerebrospinal Fluid (CSF): Evidence of Intrathecal Systnesis and Potential as Marker of Neurological Disease

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Tetranectin (TN) is a plasma protein of ~ 80 kDa. A reduction in the serum TN level has been described in malignant disease and other contitions with tissue-remodelling, whereas it does not seem to exhibit acute phase reactant behaviour. Using a polyclonal ELISA we determined the TN concentration in pairs of CSF and serum and found that the CSF/serum quotient compared to the $Q_{albumin}$ was compatible with intrathecal TN synthesis. As TN is a non covalently bound trimer in serum we characterized TN from a pool of CSF by size-exclusion chromatography, immunochemical analysis and ultracentrifugation and found results in agreement with the molecular weight previously desribed for serum TN and the molecular weight deducible from the cloned gene of the monomer. In clinically definite multiple sclerosis, but not with optical neuritis, we found a reduced CSF/serum quotient suggesting that the hypothesis of a reduced TN level as a marker of tissue remodelling may be extended to the CNS.

Diagnosis of Rhinoliquorrhoe by Detection of Transferrin (Tf) Isoforms Using Automated Isoelectric Focusing (IEF) and Immunofixation

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The determination of β_2-transferrin in nasopharyngeal secretions by agarose- or polyacrylamide-electrophoresis is commonly used for the detection of cerebrospinal fluid (CSF) fistulae. Since IEF has a higher resolution power than conventional electrophoresis, demonstrated by the detection of oligoclonal bands or of genetically determined protein variants, we developed an IEF procedure using the PhastSystem™ for the detection of Tf isoforms in human serum [1]. - By this procedure we are able to investigate three causes of Tf microheterogeneity: i. varying Tf iron saturation leading to apo-Tf, monoferric and diferric Tfs; ii. different sialic acid contents (from asialo- to heptasialo-Tf); iii. altered amino acid sequences [1]. The procedure [1] was too insensitive to analyse CSF or nasopharyngeal secretions. To increase the sensitivity we visualized the Tf bands by automated silver staining after immunofixation and removing unprecipitated material by washing with saline. To increase the separation of isotransferrins we used self-made polyacrylamide microgels (pH 5-6). - Applying these modifications to the analysis of native CSF and nasopharyngeal secretions we found asialo-Fe_2-Tf („tau fraction", „β_2-transferrin") and differently sialylated Fe_2-Tfs in CSF samples whereas in the corresponding blood serum samples only sialylated Fe_2-Tfs were detected. In some nasopharyngeal secretions small quantities of asialo-Tf, indicating a CSF contamination, were shown by IEF whereas commonly used electrophoretic procedures failed. More samples have to be analysed to confirm our findings.

In summary, our modified IEF procedure allows sensitive and specific separation of Fe_2-Tf isoforms with varying sialic acid contents in CSF, nasopharyngeal secretions and serum. For a correct diagnosis of CSF contamination a simultanous analysis of nasopharyngeal secretions and corresponding blood serum samples together with a CSF standard is inevitable.

1. Hackler R, Arndt T, Kleine TO, Gressner AM. Effect of separation conditions on automated isoelectric focusing of carbohydrate-deficient transferrin and other human isotransferrins using the PhastSystem. Anal Biochem 1995:230:281-9.

* Presented at the European CSF Symposium on Recent Progress in Cerebrospinal Fluid (CSF) and Protein Diagnosis in the Laboratory Diagnosis of Diseases of the Human Nervous System, Topic V, Marburg an der Lahn, Germany, September 29 and 30, 1995

