

C3c INTRATHECAL SYNTHESIS EVALUATION IN PATIENTS WITH MULTIPLE SCLEROSIS

Bárbara Padilla-Docal, Alberto J Dorta-Contreras, Hermes Fundora-Hernández, Elena Noris-García, Raisa Bu-Coifíu-Fanego, Marlen González-Hernández, Alexis Rodríguez-Rey

ABSTRACT - Introduction: Multiple sclerosis (MS) is a chronic, inflammatory and progressive disease of the central nervous system in which local inflammatory injuries of the brain white matter appears, being the most outstanding feature the myeline loss (demyelination). **Objective:** To determine if the complement system might be involved in the MS immunopathogeny favouring the mechanism intervening in the myelin destruction. **Method:** Samples of sera and CSF from twelve patients with a diagnosis of MS obtained at the moment of the admission to the hospital at the beginning of the break out, were collected. Levels of C3c and albumin in sera and in CSF were quantified using radial immunodiffusion plates. **Results:** High values over 80% of intrathecal synthesis were obtained except in one of the patients. **Conclusion:** Intrathecal synthesis of C3c and its liberation to the CSF means that the activation of the complement system in any of the two ways has taken place, and that once performed its biological functions, has suffered a degradation process

KEY WORDS: cytotoxicity, complement, multiple sclerosis, hypersensitivity, immunopathogenicity, reibergram.

Evaluación de la síntesis intratecal de C3c en pacientes con esclerosis múltiple

RESUMEN - Introducción: La esclerosis múltiple (EM) es una enfermedad crónica, inflamatoria y progresiva del sistema nervioso central que cursa con la aparición de lesiones inflamatorias focales en la sustancia blanca cerebral, en las que lo más llamativo es la pérdida de mielina (desmielinización). **Objetivo:** Conocer si el sistema de complemento puede estar involucrado en la inmunopatogenia de la EM favoreciendo los mecanismos que median la destrucción de la mielina. **Método:** Se colectaron muestras de suero y LCR de doce pacientes con diagnóstico de EM obtenidas en el momento del ingreso al inicio del brote. Se cuantificaron los niveles de C3c y albúmina en suero y en LCR en placas de inmunodifusión radial. **Resultados:** Se obtuvieron altos valores que superan el 80% de síntesis intratecal, menos en uno de los pacientes. **Conclusion:** La síntesis intratecal de C3c y su liberación al LCR significa que ha sucedido la activación del sistema de complemento en alguna de las dos vías y que una vez cumplidas sus funciones biológicas, ha sufrido un proceso de degradación y liberación al LCR en forma de C3c.

PALABRAS-CLAVE: citotoxicidad, complemento, esclerosis múltiple, hipersensibilidad, inmunopatogenia, reibergrama.

Multiple sclerosis (MS) is a chronic, inflammatory and progressive disease of the central nervous system (CNS) in which local inflammatory injuries of the brain white matter appears, being the most outstanding feature the myeline loss (demyelination), with a relative preservation of the axons in the precocious phase, although it may be very affected in the final phases¹. It is more frequent in young adults, although it can appear at any age. Female sex is predominant, 60% of women and 40% of men^{2,3}. Now-

adays little is known about the etiology and pathogenicity of this disease. Many authors suggested that the immune system deregulation has an important paper in the mediation of the inflammatory injuries, in the central scope. The immune misbalance in MS has been demonstrated in the cellular immune response as well as in the humoral^{4,5} and, even though there is not a known associated gene to this disease, it has been verified a clear polygenic genetic susceptibility^{6,7}.

Laboratorio Central de Líquido Cefalorraquídeo (LABCEL), Facultad de Ciencias Médicas "Dr. Miguel Enríquez", Instituto Superior de Ciencias Médicas de La Habana.

Received 16 January 2007, received in final form 21 May 2007. Accepted 29 June 2007.

Dra. Bárbara Padilla Docal - Laboratorio Central de Líquido Cefalorraquídeo (LABCEL) / Facultad de Ciencias Médicas "Dr. Miguel Enríquez" / Instituto Superior de Ciencias Médicas de La Habana - Apartado 10049 - CP 11000 Ciudad Habana - Cuba. E-mail: barbara.padilla@infomed.sld.cu

It is known that the MS diagnosis is based mainly in the clinical characteristics⁸. Cerebrospinal fluid (CSF) is normal in MS. Nevertheless, the molecular analysis shows a relative rise of the immunoglobulines, mainly the IgG. The most characteristic findings are, in frequency order: the appearance of the local IgG synthesis and the presence of oligoclonal bands in 90% of the cases, the raise of IgG in 80%, a discrete rise of gammaglobulines in 70% of the cases, and a moderate rise of cells and tubular proteins in 40% of the patients^{9,10}.

C3c is a degradation product of C3 factor of the complement system; its formation has two important biological features. One of them is that for being a product of a C3 degradation, is a measure of its concentration and, on the other hand, indicates that all C3c produced in the CNS is a product of the system biological activation in any of its ways. This last assertion means that the system has been activated and that we are in the presence of an immunological event associated to a type II or citotoxic hypersensitivity. C3c intrathecal synthesis helps us to understand the physiopathological mechanisms when we are in the presence of infectious or autoimmune neurological diseases as MS¹¹.

The objective of the present study is to know if the complement system might be involved in the multiple sclerosis immunopathogeny favoring the mechanism intervening in the myeline destruction.

METHOD

Sera and CSF samples were collected from 12 patients diagnosed with MS received in LABCEL between 2004 and 2006.

Samples were obtained at the time of the admission, at the beginning of the break out, kept at -80°C until the moment of its use in small portions.

C3c levels in sera were quantified in radial immunodiffusion plates NOR Partigen from Dade-Behring (Marburg) and in CSF in plates LC Partigen of the same brand.

To discriminate if there is C3c intrathecal synthesis and to know the situation of the blood-CSF barrier, albumin in sera was quantified in radial immunodiffusion plates NOR Partigen and in CSF in LC Partigen plates, dade Behring (Marburg).

The results obtained were placed in the Reibergram designed for C3c¹².

RESULTS

The review of the clinical files of these patients main showed that the relapsing remitting pattern was the main form of presentation of the disease and only one patient showed the primary progressive form.

Table. C3c Intrathecal synthesis and time of evolution of the disease per patient.

Patient	C3c intrathecal synthesis (%)*	Evolution time
1	89.3	2 months
2	89.5	1 year
3	87.9	3 months
4	36.5	7 months
5	93.3	4 months
6	91.2	3 years
7	92.4	18 years
8	90.1	2 years
9	83.6	5 months
10	98.7	2 years
11	96.3	15 years
12	97.4	5 years

*amount of IgG locally synthesized/ total IgG in CFSx100.

Out of the 12 patients, four were classified as MS clinically defined, six as MS defined with laboratory support, one as clinically probable MS and one as probable MS with laboratory support according Poser criteria for the MS diagnosis¹³

Regarding sex, 10 patients correspond to females (83.3%) and 2 to males (16.7%). All the patients were between 33 and 57 years old for an average of 46.8 years.

Table shows C3c intrathecal synthesis and time of evolution of the disease per patient.

Figure shows the graph of Reiber ratios or Reibergram for the evaluation of the C3c intrathecal synthesis evaluation. High values over 80% of intrathecal synthesis were obtained in all but one of the patients.

DISCUSSION

Samples used in this study come from patients diagnosed as having MS, according to the Poser criteria for the diagnosis of the disease¹³. C3c is a polypeptide structure produced by the activation and later degradation of the C3 complement factor. Antigen-antibody complex (Ag-Ab) initiates the activation of the classic way, whereas the alternative way and the lectin are independently activated from Ab through the complement interaction with specific carbohydrates groups and lypopolysaccharides present in the pathogen surface. The complement functions as a cascade through the proteolysis of a series of proteins that take to the generation of active proteins that mediate various biological functions.

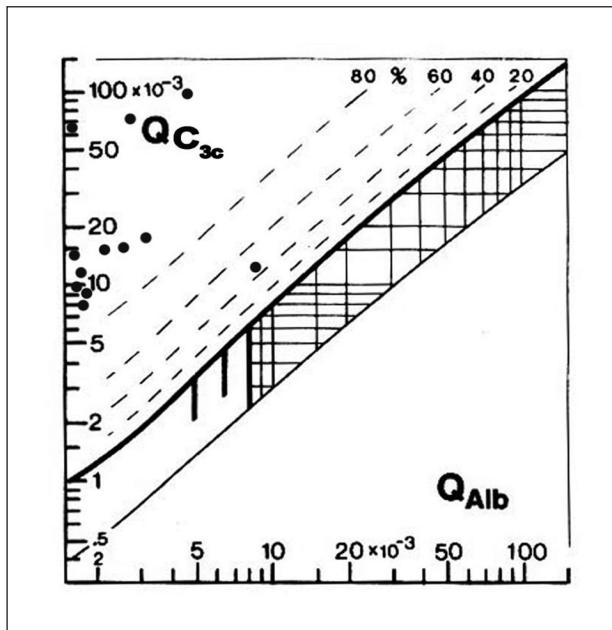


Figure. C3c Reibergram for the MS patients studied. Q_{C3c} and Q_{Alb} values are shown. Observe that there is a pronounced intrathecal synthesis in the majority of these patients because they are placed in the nearby zones to the percentile line corresponding to the 80%.

In the studied patients an intense intrathecal synthesis and C3c liberation to CSF occurred. Intrathecal synthesis of C3c and its liberation into cerebrospinal fluid means in first place that an activation of the complement system, in some of the ways it mediates, has occurred and also that a biological use of this protein took place and that after it has fulfilled its biological functions, has suffered a degradation process and liberation to CSF in form of C3c.

This allows us to sustain that a type II hypersensitivity process has occurred mainly from the synthesis of IgG that activated the complement system as part of the cytotoxicity against the myelin in the immunopathogenic mechanisms of this entity¹⁴. In this reaction; the IgG type antibodies are bonded to myelin antigens causing its destruction. These antibodies cause sickness through three principal mechanisms: opsonization and phagocytosis, antibody dependent cellular cytotoxicity and injuries mediated by the complement system. This triggers the lysis through the formation of a membrane attack complex (MAC), the attraction of the macrophages (chemotaxis) that liberate toxic substances, develop inflammatory responses and opsonization with the consequent phagocytosis¹⁵⁻¹⁷.

It has been pointed out that the complement cascade activation during the multiple sclerosis attacks seems to be reserved for the patients with the disease in advanced stages and is correlated in a significative way with the neurological incapacity of these patients¹⁶. In our study we appreciate C3c intrathecal synthesis in more than 80%, the same in patients with only few months of the disease evolution as well as in patients suffering it for several years (Table). Out of the 12 studied patients, four of them had neurological incapacity being coincident with more than 80% of C3c intrathecal synthesis.

These results allow to suggest that the complement system is involved in the immunopathogeny of MS and it will help to know much in depth the mechanisms of the myelin destruction.

REFERENCES

- Esclerosis múltiple. Bases clínicas y patológicas. In Raine CS, McFarland F, Tourtellotte WW (Eds). Madrid: Edimsa, 2000:12-18.
- Mc Farlin DE, Mc Farland HF. Multiple sclerosis. Part 1. N Engl J Med 1982;307:1183-1188.
- Mc Farlin DE, Mc Farland HF. Multiple sclerosis. Part 2. N Engl J Med 1982;307:1246-1251.
- Lassmann H. Pathology of multiple sclerosis. In Campston A, Ebers G, Lassman H, McDonald I, Matthews B, Weckerle H (Eds). McAlpine multiple sclerosis. London: Churchill Livingstone, 1998:323-358.
- Swanson JW. Multiple sclerosis, update in diagnosis and review of prognostic factors. Mayo Clin Proc 1989;64:577-586.
- Rudick RA, Ransohoff R. Cytokine secretion by multiple sclerosis monocytes. Arch Neurol 1992;49:265-270.
- Hartung HP. Immune-mediated demyelination. Ann Neurol 1993; 33: 563-567.
- Abers GC. Genetics and multiple sclerosis: an overview. Ann Neurol 1994;36(Suppl):S12-S14.
- Rao SM, Gary JL, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns and prediction. Neurology 1991;41:685-691.
- Andersson M, Alvarez-Cermeño J, Bernardi G, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol Neurosurg Psychiatry 1994;57:897-902.
- Dorta-Contreras AJ. Reibergrama para la evaluación de la síntesis intratecal de C3c. Barrera sangre-líquido cefalorraquídeo. La Habana: Editorial Academia, 2006:69-67.
- Dorta-Contreras AJ, Noris-García E, Padilla-Docal B, et al. Reibergrama para la evaluación de la síntesis intratecal de C3c. Arq Neuropsiquiatr 2006;64:585-588.
- Poser CM, Paty DW, Scheinberg L et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13: 227-231.
- Stangel M, Compston A, Scolding NJ. Oligodendroglia are protected from antibody-mediated complement injury by normal immunoglobulins. J Neuroimmunol 2000;103:195-201.
- Lucchinetti C, Brück W, Noseworthy J. Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment. Curr Opin Neurol 2001;14:259-269.
- Rodríguez-Sainz MC, Sánchez-Ramón S, de Andrés C, Rodríguez-Mahou M, Muñoz-Fernández MA. Th1/Th2 cytokine balance and nitric oxide in cerebrospinal fluid and serum from patients with multiple sclerosis. Eur Cytokine Netw 2002;13:110-114.
- Sellebjerg F, Jaliashvili I, Christiansen M, Garred P. Intrathecal activation of the complement system and disability in multiple sclerosis. J Neurol Sci 1998;157:168-174.

CORREÇÃO / CORRECTION

C_{3c} intrathecal synthesis evaluation in patients with multiple sclerosis

B Padilla-Docal, A J Dorta-Contreras, H Fundora-Hernández, E Noris-García, R Bu-Coifiu-Fanego, M González-Hernández, A Rodríguez-Rey

Arq Neuropsiquiatr 2007;65(3B):800-802

Figure of this article is wrong. Correct Figure is

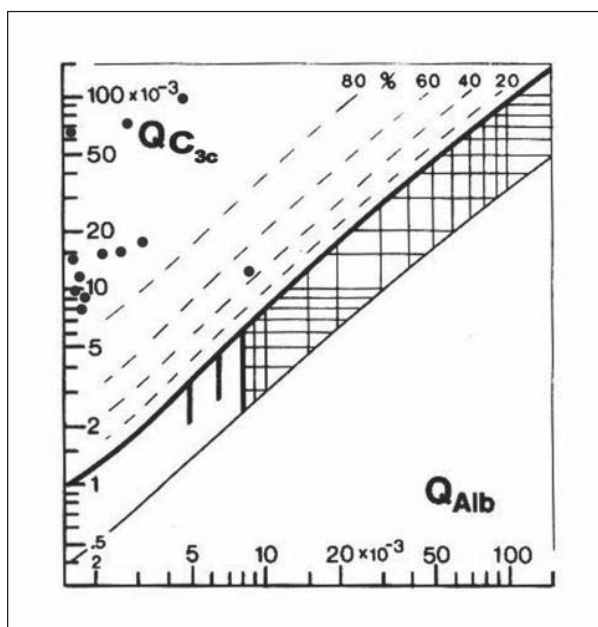


Figure. C_{3c} Reibergram for the MS patients studied. Q C_{3c} and Q Albumin values are shown. Observe that there is a pronounced intrathecal synthesis in the majority of these patients because they are placed in the nearby zones to the percentile line corresponding to the 80%.

The Editor presents to the Authors the excuses for this distressing mistake.

Antonio Spina-França

This article has received corrections in agreement with the ERRATUM published in Volume 65 Number 4b.