REPORT

Pattern of intrathecal immunoglobulin synthesis in pediatric patients with infectious meningoencephalitis

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ABSTRACT

Intrathecal immunoglobulin synthesis is part of the immune response in the central nervous system (CNS). In this study we demonstrate that age, the features of the biological agent, and epidemiological factors modulate the pattern of intrathecal immunoglobulin (Ig) synthesis. We also describe the pattern of intrathecal Ig in patients suffering from bacteria, virus, or parasites induced meningoencephalitis is the general objective of the study. The description of the pattern of intrathecal Ig and IgG subclasses in children with eosinophilic meningoencephalitis due to *Angiostrongylus cantonensis* and study the pattern of intrathecal immune response and the functionality of the blood-cerebrospinal fluid (CSF) barrier in children suffering from meningoencephalitis due to *Neisseria meningitidis*, *E*chovirus 4, 6, 9, 16 and Coxsackie A_9 and B_5; and propose novel applications and modifications of reibergram,. Each one of the infectious diseases studied exhibit a characteristic pattern of intrathecal immunoglobulin synthesis. These patterns are detected earlier in children than in adults with similar infections. Additionally, significant differences were found in the Ig patterns during epidemics as compared to non-epidemic infections. The reibergram is therefore a useful tool in the characterization of epidemics. The reibergram was also adjusted for IgG subclasses, being especially relevant for IgG3. The pattern of intrathecal Ig for these diseases in children is reported here for the fist time. The novel use of reibergrams in the evaluation of IgG subclasses opens new avenues for neuroimmunology in general and particularly for pediatric and tropical neuroimmunology.

*I*ntroduction

Neuroimmunology was founded in the last decades of the XX century as a science bridging neurology and immunology. The analysis of the cerebrospinal fluid (CSF), together with the application of imagenological techniques has been helpful to understand the molecular events taking place inside the brain [1, 2]. However, relevant studies on intrathecal immunoglobulin synthesis in children with transmissible diseases are lacking. These diseases, which are often epidemic, are an important world health problem, and Cuba is no exception.

Several studies on intrathecal immunoglobulin synthesis have been conducted in adults and, as a rule, they were not related to epidemics [3-5], but it was taken for granted that a uniform pattern existed for all viral meningoencephalitis, except herpetic meningoencephalitis [6, 7].

We hypothesize that age, the features of the biological agent, and epidemiological conditions modulate the patterns of intrathecal immunoglobulin synthesis. To demonstrate this hypothesis a reibergram was adapted to evaluate the intrathecal synthesis of IgG_3 and dilucidate the epidemiological value of its patterns.

*M*aterials and methods

Children with A*ngiostrongylus cantonensis*, *Neisseria meningitidis*, Echovirus 4, 6, 9, and 16 and Coxsakie A_9 and B_5 mediated epidemic and non-epidemic meningoencephalitis were studied. Patients studied under epidemic conditions (80) were classified as follows: Echovirus 4 in 1985, 84 patients; Echovirus 6 in 1995, 43 patients; Coxsackie B5 in 1994, 48 patients; Coxsackie A9 between 1993 and 1994, 23 patients; Echovirus 9 in 1999, 31 patients; and Echovirus 16 in 2000, 18 patients.

Serum and CSF samples were taken from these subjects, and IgA, IgM, IgG and bovine seroalbumin contents in both biological fluids were quantitatively determined by nephelometry or radial immunodiffusion. IgG subclasses were assessed by radial immunodiffusion.

Since IgG_3 exhibits a higher molecular mass and different molecular structure as compared to IgG_1, IgG_2 and IgG_4, the reibergram was modified for this immunoglobulin subclass. A general reibergram was conducted for each biological agent, in either epidemic or non-epidemic situations.

*R*esults

The pattern of intrathecal immunoglobulin synthesis was higher in children with meningoencephalitis. All children lived in the same geographic area in Havana and were infected by different microorganisms (Table 1).

The pattern of intrathecal Ig was reported here for the first time in a parasite induced CNS disease: the eosinophilic meningoencephalitis due to *Angiostrongylus cantonensis* (figures 1 and 2).

No intrathecal Ig synthesis was found at the first lumbar puncture; however, dysfunction of the blood-CFS barrier was documented. During the second lumbar puncture, the three IgG subclasses and IgA were detected in the CSF. The pattern was $IgG_1 + IgG_2$.

A different pattern of intrathecal immunoglobulins was found in epidemic viral meningoencephalitis as compared to non epidemic infections.

Additionally, the pattern of intrathecal immunoglobulins in patients with Echovirus 4 and 6, and Coxsackie A_9 and B_5 were reported here for the first time.

An IgA dependent pattern was found in adult patients with *Neisseria meningitidis* induced

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Table 1. Patterns of intrathecal immunoglobulin synthesis (first puncture)

Biological agent	Epidemic synthesis patterns (frequency)	Inter-epidemic synthesis patterns (frequency)
Echo 4	IgA+IgM+IgG (74/84) IgA+IgG (10/84)	IgA+IgM+IgG(7/8) IgAS+IgG(1/8)
Echo 6	IgG (42/43) IgA+IgG (1/43)	IgA+IgM+IgG (3/10) IgA+IgG (7/10)
Echo 9	Without synthesis (15/23) IgM (6/23) IgA+IgM (2/23)	-
Echo 16	Without synthesis (16/18) IgA+IgM (2/18)	-
Coxsackie A9	IgM(5/31) IgG (2/31) IgA+IgM(2/31)IgG+IgM (1/31)sin síntesis (20/31)	IgA+IgM+IgG (7/10) IgA+IgG (2/10) IgG (1/10)
Coxsackie B5	IgA+IgM+IgG(14/48) IgA+IgM (5/48) IgM+IgG (2/48) IgG (17/48) Without synthesis (10/48)	IgA+IgM+IgG (6/8) IgA+IgG (1/8) IgG(1/8)
Neisseria meningitidis	-	IgA+IgM+IgG (9/26) IgA+IgG (12/26) IgA (2/26) Without synthesis (3/26)
Angiostrongylus cantonensis	-	Without synthesis (0/24) Without synthesis (0/10)

meningoencephalitis. Intrathecal immunoglobulin synthesis was detected in children suffering from bacterial and viral meningoencephalitis from the first lumbar puncture.

The reibergram was a valuable epidemiological tool, because changes in its frequency or pattern could be related to structural changes or different clinical symptoms in the patients.

The intrathecal Ig pattern against Echovirus was similar in epidemic or non epidemic samples, but this was not true for the other viruses studied (Figure 3).

Next, we analyzed the frequencies of the three-class, two-class and one-class pattern, without considering the class of Ig involved. No differences between epidemic and non-epidemic situations were found, stressing the relevance of the qualitative evaluation of the immunoglobulin class.

However, the differences in the immunoglobulin patterns between epidemic and non epidemic situations were confirmed using the Chi square test, f coefficient, and odds ratio.

The adaptation of reibergram to IgG_3 was an important methodological result, because it helped us characterize the pattern of intrathecal Ig synthesis. Due to the molecular features of this subclass a particular reibergram, different from the other IgGs was required.

The novel formula used to calculate the levels of local IgG_3 was compared to other formulas used for the same purpose. Identity among results of Link index, IgG_3 reibergram and the novel reibergram was observed in patients with Echovirus 6 induced viral encephalitis. This result, together with the biological advantages of the novel reibergram, based on the molecular characteristics and molecular diffusion

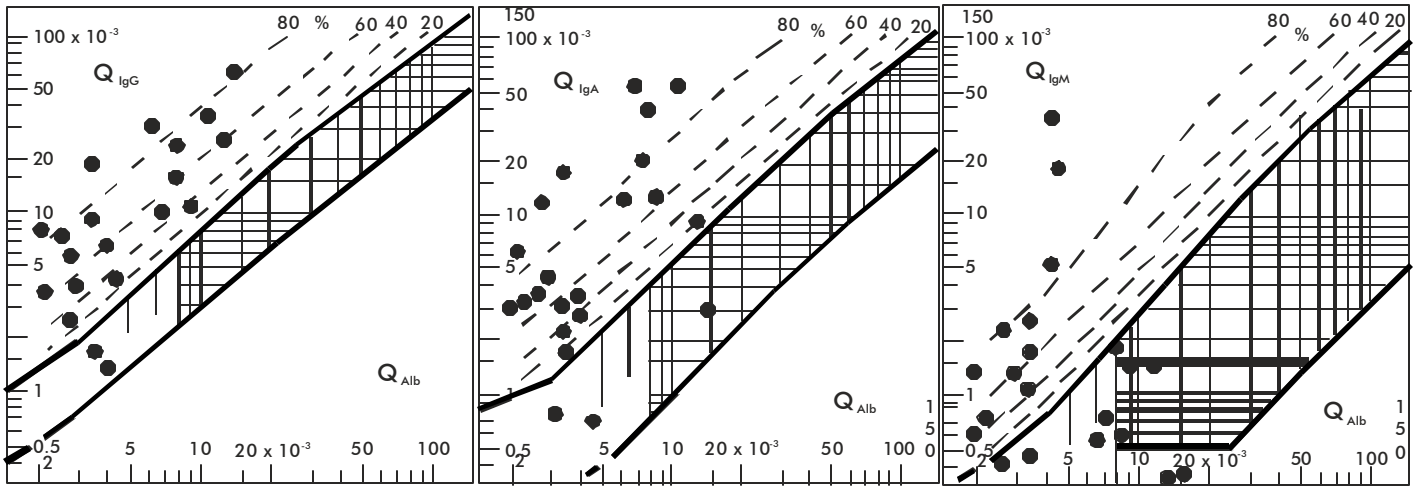

Figure 1. Reibergram of intrathecal immunoglobulins in patients with meningoencephalitis due to *Angiostrongylus cantonensis*. A Q/Ig above the stronger hyperbolic curve indicates intrathecal synthesis of this protein. A predominant intrathecal synthesis of IgG and IgA is observed.

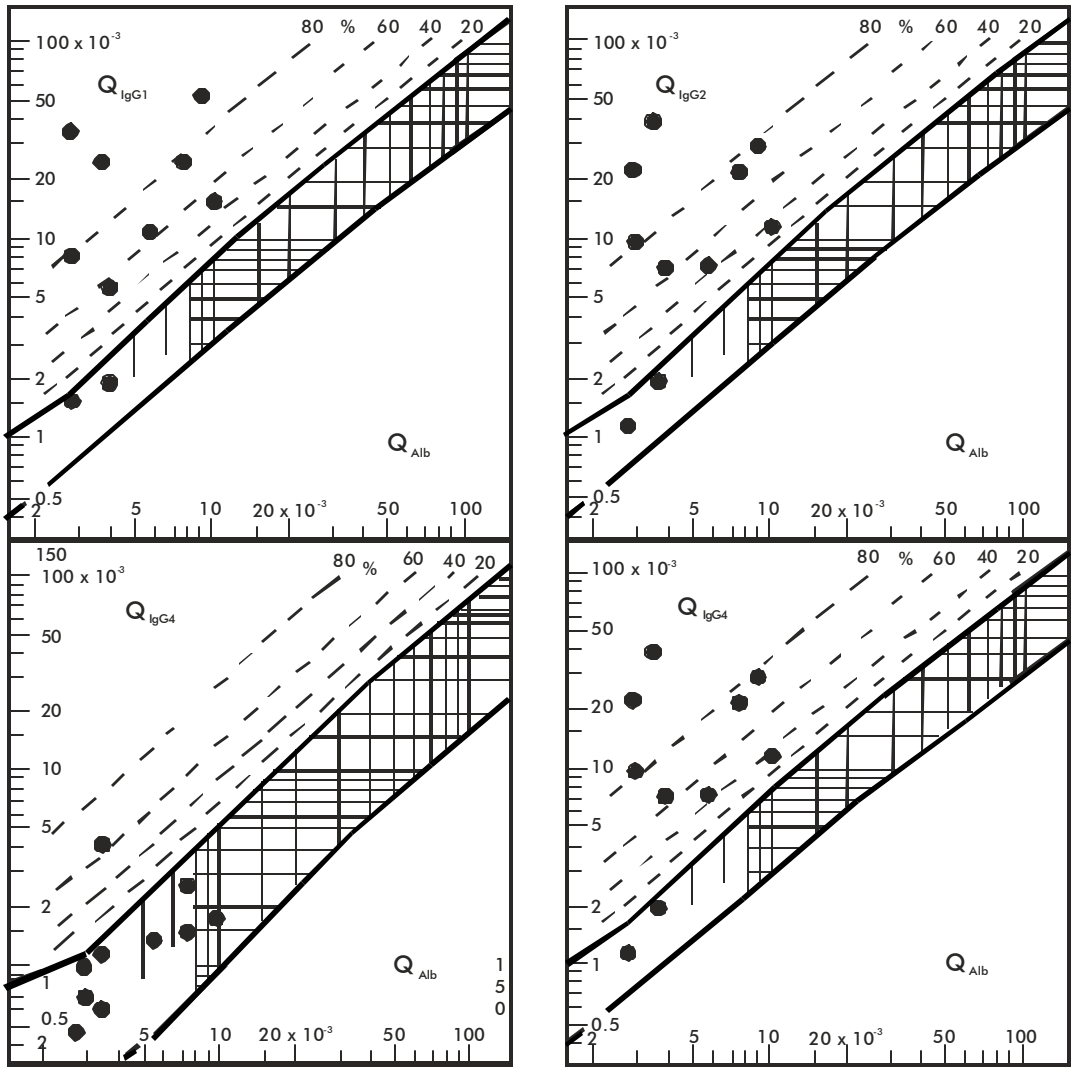

Figure 2. Reibergram for IgG subclass synthesis in *Angiostrongylus cantonensis* patients. A predominant IgG_1 + IgG_2 pattern was observed.

theory of r/CSF flow, lead to the conclusion that the proposed methodology is more effective than that previously used.

*D*iscussion

Nine articles containing partial results of this thesis were published in high impact international journals, and included in the Medicus/Medline Index during the last five years [8-16] and in annexes [17-20].

The following results were reported here for the first time:

1. The description of the pattern of intrathecal immunoglobulin synthesis and IgG subclasses in a parasitic disease.

2. The demonstration that intrathecal immunoglobulin synthesis differs among patients with viral meningoencephalitis, and even for the same virus it is different in epidemic compared with non-epidemic infections.

3. The description of the pattern of intrathecal immunoglobulin synthesis in patients with viral meningoencephalitis induced by Echovirus 4, 6, 9 and 16 and by Coxsackie A_9 and B_5.

4. The demonstration that intrathecal immunoglobulin synthesis in children with viral and bacterial meningoencephalitis can be detected at the first diagnostic puncture.

5. The epidemiological contribution of reibergram values, which links the changes observed in the pattern of intrathecal immunoglobulin synthesis, with the structural mutations and modifications in the clinical behavior of patients with viral meningoencephalitis.

6. The adaptation of reibergram for an accurate detection of intrathecal IgG_3 synthesis.

The study of cerebrospinal fluid started in 1891, when the first lumbar puncture with diagnostic and therapeutic purposes was carried out by Quincke.

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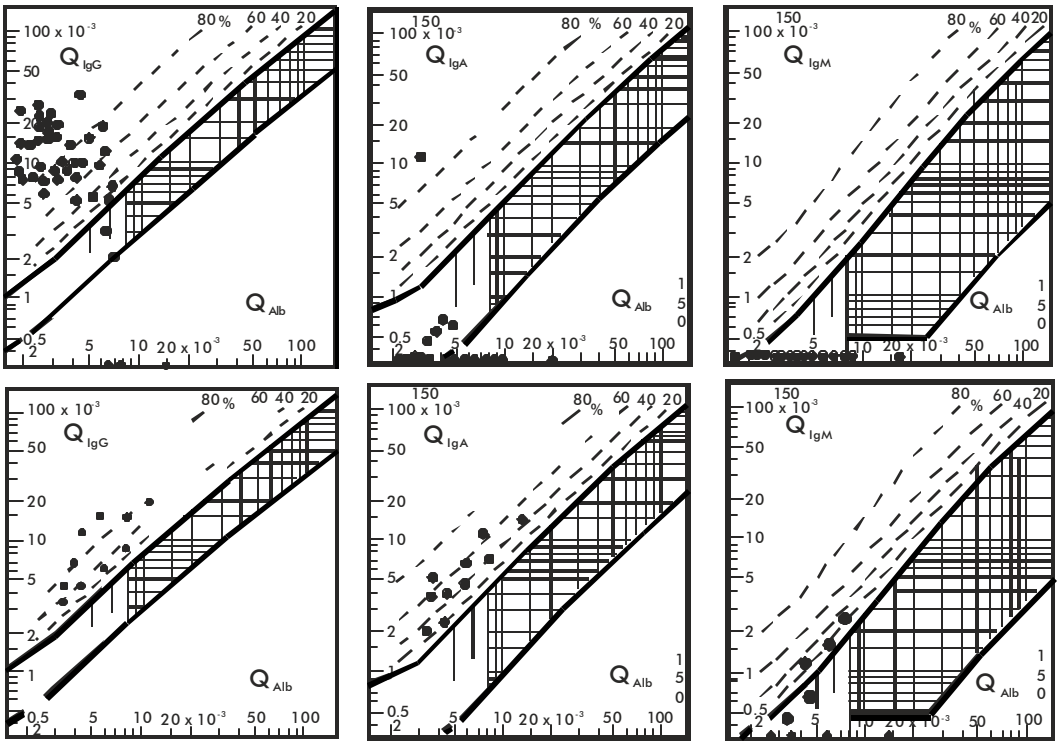

Figure 3. Reibergrams from Echovirus 6 mediated meningoencephalitis in outbreaks (a) and non epidemic situations. Intrathecal IgG synthesis was predominant; only one patient synthesized IgA and none IgM; therefore the pattern differed from those observed in the other reibergram.

The antibodies secreted by B lymphocyte-derived immunoglobulin-secreting plasma cells in the central nervous system are a pattern of intrathecal immunoglobulin synthesis. Those cells are abundant in the CSF, where they can be readily detected.

The reibergram is a useful tool for the diagnosis of neurological disorders. It offers relevant information on blood/CSF barrier, by monitoring intrathecal Ig levels and estimating the proportion of these proteins synthesized in the central nervous system (intrathecal fraction). Each disease is associated to a characteristic pattern of intrathecal immunoglobulin synthesis; therefore the knowledge of this parameter can contribute to disease diagnosis.

In addition to other tests and clinical observations, the pattern of intrathecal Ig synthesis can rule out a group of diseases during diagnosis. It will be possible to diagnose a disease based on the antibody index, or to characterize, at an early stage, chronic inflammatory processes such as multiple sclerosis. It can also help to evaluate the efficacy of therapies, and to better understand the organic course of the brain in psychiatric diseases, among other uses.

The reibergram can also offer basic and theoretical knowledge on the physiopathology of neurological diseases.

Angiostrongylus cantonensis derived Meningoencephalitis (Chen, 1935), was reported for the first time in Cuba in 1981. The pattern of intrathecal immunoglobulin synthesis in children with parasitic disease affecting the central nervous system was studied for the first time in 1998 [8].

After the work with *A. cantonensis*, we studied the pattern of intrathecal Ig synthesis in African trypanosomiasis patients [21, 22]. The pattern of intrathecal Ig synthesis in patients infected with serogroup B *Neisseria meningitidis*, a pathogen responsible for many outbreaks until Cuban scientists developed the only effective vaccine available, were also described [23-25].

IgA, which initiates the process of bacterial killing, is an important component of the pattern [26-28]. The composition of the pattern is identical in children and adults, as reported elsewhere [29]. We found, however, that intrathecal Ig synthesis can be detected in children during the first diagnostic lumbar puncture. This is a new finding since previous studies in adults reported that no intrathecal Ig can be detected at the onset of clinical symptoms [30].

The average number of cells in the CSF is $20/10^{-6}$ L in children and $5/10^{-6}$ L in adults [29]. The immature nature of the blood/CFS barrier in children has been invoked to explain these differences. [31] However, the theoretical bases of the mechanisms of molecular diffusion and flow rate through CSF confirm that those barriers are well structured and defined since embryogenesis.

The entrance of cells from the blood to the subaracnoideal space can be alternatively explained by a shorter distance from the blood compartment to the subaracnoideal space in children compared to adults. This traffic is enhanced during the inflammatory processes characteristic of many infections. In children, the accelerated influx of

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activated B lymphocytes allows an earlier detection of intrathecal Ig. Moreover, the flow rate of the CSF is slower in children, especially during the first years of age when high levels of Q albumin are found, and the effect of bacterial infections on the central nervous system is noteworthy.

According to the theory, a lower CSF flow rate, and an increased number of molecules and cells entering the CSF from the blood stream are consequences of the elevated Q albumin levels [17].

A common approach to the study of the patterns of intrathecal Ig synthesis in viral meningoencephalitis, with the exception of mumps and herpes virus, can be found in the literature. However, every viral agent induces a typical intrathecal Ig pattern, depending on the characteristics of the immune response elicited.

The patterns of intrathecal Ig synthesis in Echovirus 4, 6, 9 and 16, and Coxsackie B_5 and A_9 induced meningoencephalitis during epidemic and non-epidemic periods reflects the features of the immune response, which are closely related to the clinical condition of these infections. These are the bases for the usefulness of the reibergram in the study and in the early diagnosis of different infections. Each epidemic leaves its footprint as a unique pattern in the reibergram, which is often different from the pattern found in non-epidemic infections.

Recent studies have concluded that enteroviruses, particularly Coxsackie, can experience structural changes after infecting a stressed host [32, 33]; possibly due to micronutrient deficiency. Different strains of modified viruses are generated after these changes, which can in turn cause different diseases. This seems to be the case of Coxsackie A_9, as reported elsewhere [34-36].

The value of the reibergram as an epidemiologic tool is supported by the experience gathered in the neuroimmunological studies conducted for more than 15 years in the population receiving medical care in San Miguel del Padrón Pediatric Hospital in Havana.

The pattern found in this population during the course of the epidemic reflects the multiple interactions produced during the health/disease process.

The usefulness of the reibergram is enhanced by the organization and centralization of the Cuban health system, which guarantees unrestricted access to diagnosis for every patient as well as specialized medical treatment from the very onset of the clinical symptoms. At the same time, the systematic collection of reliable information is greatly facilitated by this organization [12].

Another important objective of this investigation was the application of the reibergram to the quantitative detection of the intrathecal synthesis of IgG subclasses. In this sense, IgG_3 exhibits molecular characteristics which distinguish it from the rest of the IgG subclasses. Its molecular mass is higher, therefore its diffusion towards the subaracnoideal space is slower, according to Fick's first law ($J_i = -Dc_i/dx_i$). The diffusion coefficient (D) depends on the molecular mass and affects CSF flow rate (F).

The novel IgG_3 reibergram solved the drawbacks of the traditional reibergram, leanding to an accurate estimation of the fraction of this subclass synthesized within the CNS [13].

The IgG_1 + IgG_2 pattern of synthesis prevailing in *Angiostrongylus cantonensis* associated meningoencephalitis indicates the antigenic complexity of the immune response against this parasite; because IgG_1 is mainly responsible for the response against proteins, while IgG_2 is generally directed against polysaccharide antigens. This combined immune response could explain the self limiting nature of the disease in the patients studied [16].

These findings could modestly contribute to the development of Neuroimmunology in general, and, particularly, to pediatric and tropical Neuroimmunology.

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