Review: Intrathecal Activation as a Typical Immune Response within the Central Nervous System in Angiostrongyliasis

Barbara Padilla-Docal,* Ivonne Iglesias-González, Raisa Bu-Coifiu-Fanego,
Carmen Aleida Socarrás-Hernández, and Alberto Juan Dorta-Contreras
*Central Laboratory of Cerebrospinal Fluid, Faculty of Medical Sciences Dr. Miguel Enriquez,
Havana Medical Sciences University, Havana, Cuba*

Abstract. *Angiostrongylus cantonensis* is a zoonotic pathogen that occasionally causes human angiostrongyliasis; its main clinical manifestation is eosinophilic meningitis. This report defines the concept of intrathecal activation of complement as evidence of intrathecal synthesis of major immunoglobulins during this disease. Details are presented of the activation of complement system components in cerebrospinal fluid, and their application to our understanding of this tropical disease, which is emerging in the Western hemisphere. Intrathecal synthesis of at least one of the major immunoglobulins and a wide spectrum of patterns may be observed. Although intrathecal synthesis of C3c is always present, C4 intrathecal synthesis does not occur in every patient. The diversity of intrathecal synthesis and activation of the different complement pathways enables their division into three variant groups (A, B, and C). Variant group A includes the classical and/or lectin pathway and involves two or more major immunoglobulins with C3 and C4 intrathecal synthesis. Variant group B involves C4 in cerebrospinal fluid that comes from blood in the intrathecal activation of the classical pathway. Variant group C includes the alternative pathway.

INTRODUCTION

Angiostrongylus cantonensis is a zoonotic pathogen that occasionally causes human angiostrongyliasis; its main clinical manifestation is eosinophilic meningitis. The nematode worm *A. cantonensis* was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou, China.[1]

Angiostrongylus cantonensis is endemic to southern Asia,[2] the Pacific islands,[3] and the Caribbean islands,[4] and probably in South America.[5,6] The life cycle of this nematode involves rats as definitive hosts; molluscs as intermediate hosts; and crustaceans (prawns and land crabs), predacious land planarians, frogs, and monitor lizards as paratenic (transfer or transport) hosts. Humans acquire the parasite after eating intermediate or paratenic hosts, or vegetables that contain infective larvae (the third stage) of the worm.[1]

Cerebral angiostrongyliasis is generally diagnosed from the patient's clinical history and cerebrospinal fluid (CSF) eosinophilia, and is supported by a history of possible exposure to infective larvae. The gold standard in diagnosis, although often difficult, is detection of the disease agent. Angiostrongyliasis should be considered a human disease that is sometimes fatal. Frequently, the infection occurs in outbreaks with a variable number of cases.[7–9] Human angiostrongyliasis was first reported in the Western Hemisphere in Cuba in 1981,[10] and then in Haiti, Jamaica,[11] Brazil,[12] Dominican Republic,[13] and most recently in Ecuador.[14]

Complement, a central component of the innate immune system, is comprised of at least 35 proteins that collaborate in an intricate manner to eliminate microorganisms and remove apoptotic cells. Complement also serves as a natural adjuvant, enhancing and directing the adaptive immune response.[15] Although the final effect of activation of this system is the destruction of microorganisms, it may also lead to host tissue damage. The complement system has been regarded as being comprised of three activation pathways: 1) the classical path-

way initiated by C1q, 2) the lectin pathway initiated by mannan-binding lectin (MBL), and 3) the alternative pathway, which is triggered by spontaneous C3 hydrolysis or direct pattern recognition of properdin.[15,16]

It was recently demonstrated that in murine experimental cerebral malaria, the classical or alternative complement pathways are not required for disease development,[17] apparently because activation of C5 occurs via coagulation enzymes of the extrinsic protease pathway. This pathway should be considered a fourth complement pathway.

The use of Reibergrams (immunoglobulin CSF/serum quotient Reiber diagrams) has been reported for angiostrongyliasis and other infectious diseases.[18,19] Complement component C3c is a degradation and stable product of C3; it is currently quantified instead of the whole C3 molecule because its presence indicates that C3 was biologically used in the immune response. The use of C3c and C4 Reibergrams has also been reported;[20,21] these are used to confirm that local complement factor was synthesized in the central nervous system (CNS).[20,21] The validity of this option is supported by the molecular flux/CSF flow theory.[22,23] Recently, different major immunoglobulin patterns in patients with angiostrongyliasis patients in Ecuador and Cuba with dissimilar neuroimmunologic and epidemiologic features, have been reported.[5,6] Other reports described intrathecal synthesis patterns of major immunoglobulins[24] and C3 and C4 in patients with angiostrongyliasis.[20,21]

This report reviews the different patterns of this immune response and defines the concept of intrathecal activation of complement. It also aims to explain the relevance of these patterns during oligoclonal intrathecal synthesis, as well as the activation in CSF of one or more complement system components in meningoencephalitis caused by *A. cantonensis*.

Reibergrams. Reference to CSF/serum quotient diagrams or Reibergrams (Figure 1) enables an understanding of the intrathecal synthesis findings seen in angiostrongyliasis. The Reibergram was introduced for quantification of major immunoglobulin intrathecal synthesis,[24] IgG subclasses,[25] IgE,[26] and some components of the complement system.[20,21]

This procedure is based on molecular diffusion/CSF flow theory; its fundamental principle is that a decrease in flow

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*Address correspondence to Barbara Padilla-Docal, Ramon Pinto 202, Luyanó, Havana 10700, Cuba. E-mail: barbara.padilla@infomed.sld.cu
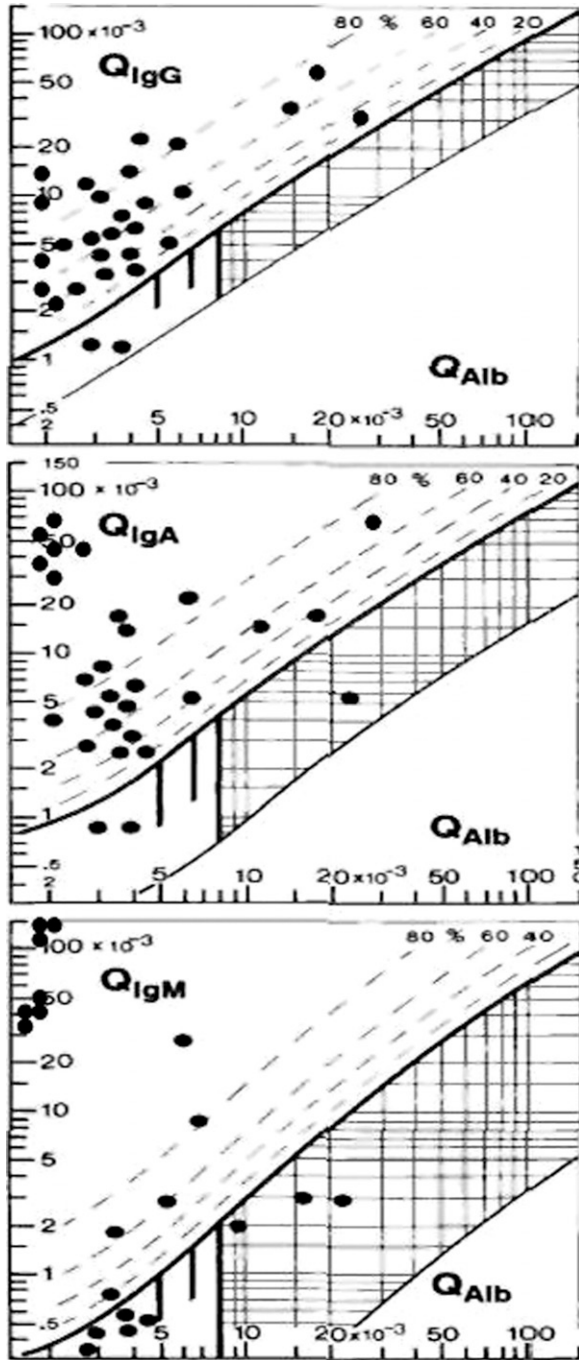
FIGURE 1.   Reibergram of major immunoglobulins in patients with eosinophilic meningoencephalitis caused by *Angiostrongylus cantonensis*.[24] Copyright by the American Society for Microbiology.

rate is always accompanied by an increase in molecular diffusion from blood to CSF. Reibergrams are diagrams that analyze in an integrated way the function of the blood-CSF barrier and intrathecal protein synthesis. They also aid in the diagnosis of CNS diseases associated with specific patterns of immunoglobulin response.[22,23]

The upper hyperbolic curve (thick line) represents the discrimination line between brain-derived and blood-derived proteins. Values above this upper line represent intrathecal specific protein synthesis such as major immunoglobulins (Figure 1), C3c (Figure 2), or C4 (Figure 3).
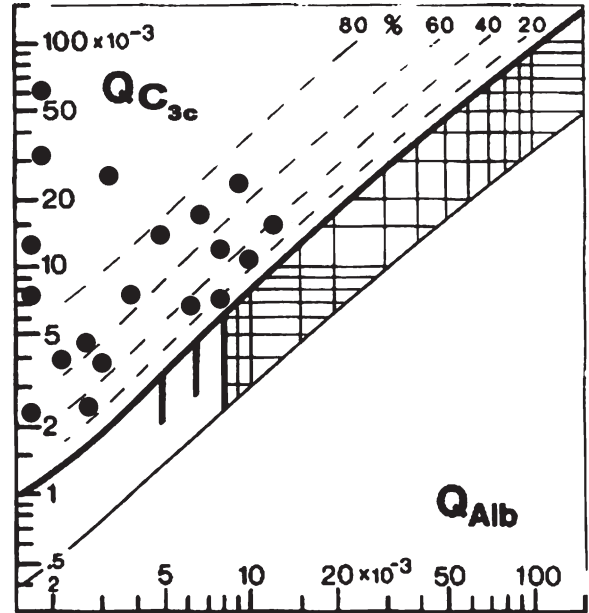

FIGURE 2.   Reibergram for C3c in patients with meningoencephalitis caused by *Angiostrongylus cantonensis*.

The dashed lines indicate the extent of intrathecal synthesis as intrathecal fractions (C4IF, C3cIF, or immunoglobulin IF) with 20%, 40%, 60%, and 80% of the measured total specific protein concentration in CSF, with reference to the discrimination line as 0% intrathecal synthesis. The limit of the reference range for QAlb between reference (normal) and increased CSF protein concentrations caused by blood-CSF barrier dysfunction is indicated by the age-dependent vertical lines: At QAlb = 5×10^{-3} ($\leq$ 15 years), at QAlb = 6.5×10^{-3} ($\leq$ 40 years), and at QAlb = 8×10^{-3} ($\leq$ 60 years). Values below the lower hyperbolic line in range 5 indicate a faulty method.[22,23]
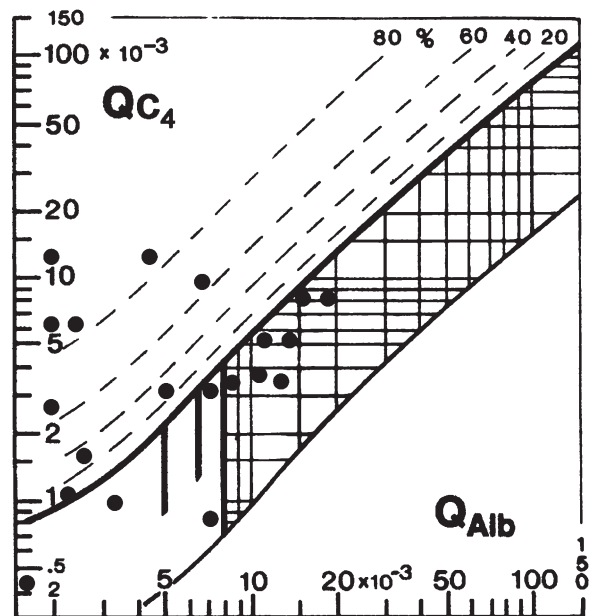

FIGURE 3.   Reibergam for C4 in cerebral angiostrongyliasis.

Pattern	Synthesis pattern	Frequency
I	IgA + IgG + IgM + C3c + C4	4/20
II	IgA + IgG + IgM + C3c	3/20
III	IgA + IgM + C3c	1/20
IV	IgA + IgM + C3c + C4	2/20
V	IgA + IgG + C3c + C4	2/20
VI	IgG + IgM + C3c + C4	1/20
VII	IgM + C3c	3/20
VIII	IgA + C3c	2/20
IX	C3c	2/20

Patterns of synthesis of intrathecal immunoglobulins and complement components C3c and C4 in angiostrongyliasis. Different patterns of intrathecal immunoglobulin synthesis have been described.[24] Diagnostic examination of CSF from the lumbar puncture shows no intrathecal IgG, IgA, or IgM class response. A week later, at the time of early clinical recovery, intrathecal immunoglobulin synthesis has emerged as either a two-class or three-class response.

Intrathecal synthesis patterns and their frequency are shown in Table 1. Pattern 1 is the most frequent and includes the major complement components C3c and C4. Intrathecal
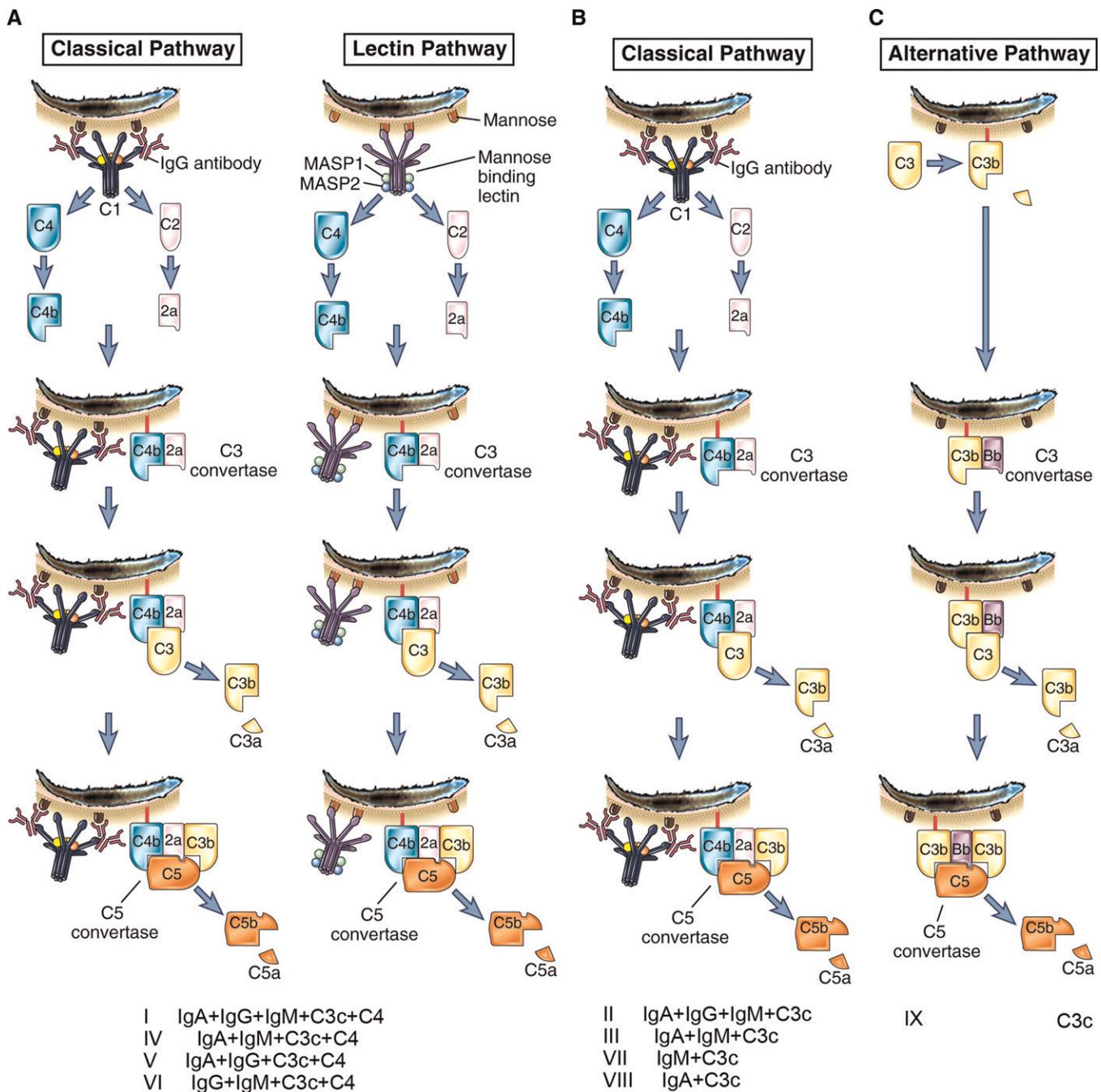

FIGURE 4.   General intrathecal synthesis variants of intrathecal patterns. Four pathways are shown.

synthesis of at least one of the major immunoglobulins may be observed in these patients; a wide spectrum of patterns may occur. As might be expected, the patients have C3c intrathecal synthesis; C4 intrathecal synthesis was noted in some patients. The C3c and C4 Reibergrams from patients with angiostrongyliasis are shown in Figures 1 and 2, respectively.

General intrathecal synthesis variants. Diversity of intrathecal synthesis and activation of different complement pathways enables their division into three variant groups (A, B, and C) (Figure 4). Samples of CSF and serum were obtained from patients diagnosed with *A. cantonensis* eosinophilic meningitis. The patients had typical symptoms of meningitis. Samples obtained at the time of admission (at the onset of symptoms) were kept in small aliquots at −80°C until analysis. All data were stored in historical records.

Variant A. This variant includes classical and/or lectin pathways and generally involves ≥ 2 major immunoglobulins with intrathecal synthesis of C3 and C4. Patterns I, IV, V, and VI were observed among this variant. Pattern I is intrathecal synthesis of major immunoglobulins with C3 and C4 plus local synthesis. The classical and/or lectin pathway (intrathecal activation) may be observed and have a potential role in the destruction of third-stage parasite larvae. Pattern IV is intrathecal synthesis of IgA and IgM simultaneously with C3 and C4 local synthesis involved in classical and/or lectin pathway (intrathecal activation). Pattern V is intrathecal synthesis of IgA and IgG simultaneously with C3 and C4 local synthesis, acting as classical and/or lectin pathway (intrathecal activation). Pattern VI is intrathecal synthesis of IgM and IgG simultaneously and can produce classical and/or lectin intrathecal activation with C3 and C4 intrathecal synthesis.

Variant B. All responses classified in this variant group are characterized by C4 in CSF that comes from blood in the intrathecal activation of the classical pathway. This group includes patterns II, III, VII, and VIII. Pattern II is classical intrathecal activation by IgG and IgM, and occasionally IgA1 may act by fixing complement with C3 intrathecal synthesis. Pattern III is classical intrathecal activation by IgM and occasionally by IgA1 with C3 intrathecal synthesis. Pattern VII shows efficient fixation of complement by IgM and intrathecal activation of C3 by the classical pathway. Pattern VIII shows intrathecal activation by a classical pathway caused by C3c and IgA1 synthesized locally.

In patients who show major immunoglobulin intrathecal synthesis but no C4 intrathecal synthesis, unique activation might be the classical pathway. However, C4 originates from blood by simple diffusion.

Variant C. This variant only includes the alternative pathway. There is only intrathecal synthesis of C3 (pattern IX). The patient included in this group show only C3c intrathecal synthesis, and the only complement pathway observed is the alternative pathway. In this pathway, the interaction among C3, properdin, and B and D factors facilitate C3bBbP complex formation, and activity against and lysis of larvae. Until now, there has been no report of C3 deficiency in patients with eosinophilic meningoencephalitis caused by *A. cantonensis*.

In the genetic absence of C3, thrombin substitutes for C3-dependent C5 convertase. This linkage between the complement and coagulation pathways may represent a new pathway of complement activation.[27] Thrombin can function as C5 convertase and provides evidence for a direct linkage between complement and clotting pathways. This finding suggests a new pathway of complement activation in the absence of C3. This recently described pathway of complement should be taken into account in future studies.

Pattern IX is characterized by intrathecal activation of the alternative pathway and absence of intrathecal synthesis of immunoglobulins. This finding suggests that intrathecal immunoglobulins are not involved in local activation of the complement system. Other complement pathways, should they be activated, will have been produced by immunoglobulins from blood.

Role of complement intrathecal activation in larval destruction. Two processes involving complement occur separately, but have an intimate relationship: biological synthesis and activation. In blood, these processes also occur separately. However, in blood they constitute a system with many possibilities. These possibilities include supplying components to different pathways capable of acting against a wide range of invading organisms. These complement components can be used variably against different microorganisms as part of the innate immune response. Complement components of different pathways act as acute-phase proteins and participate in the initial host response before immunoglobulin production.

Third-stage larvae are usually destroyed in human CSF, in which case the complement system may play an important role. To understand this local process, it is important to clarify the meaning of intrathecal activation. This term can be defined as the activation of any complement pathway mediated by one or more of the components of the complement system previously synthesized in CSF. It is important to note that intrathecal activation does not exclude the possibility that the complement system can be activated by components in blood. However, this activation is not a direct consequence of the presence of third-stage larvae of *A. cantonensis* in CSF.

Intrathecal immune response patterns and consequences for blood-CSF barrier function, combined with synthesis and activation of the complement system caused by parasites, have not been reviewed. These patterns are discussed for pathophysiologic (diagnostic and theoretical) reasons. The main feature of intrathecal activation is that it is a more specific response that occurs in CSF, in which third-stage larvae are inactivated, and in which an inflammatory response is produced. These responses are part of the process of elimination of parasites in this environment. It is noteworthy that the role of complement and its role in *in vivo* during helminth infection is poorly understood.[28] In the absence of intrathecal activation, any larvae that had evaded destruction in the blood, could migrate to the CNS. Thus, the complement system could be one of the most important elements in the brain's primary defense against infections, especially early when specific antibodies have not been synthesized.

Although immunoglobulins commonly pass from the blood to the CSF, it is nevertheless important to recognize the existence of local synthesis and the intrathecal immune response, and to determine the role played by these immunoglobulins against third-stage larvae *in situ*. In addition, local complement synthesis in the CSF is proof of production and intrathecal activation of C3 from its degradative derivative C3c.

Peculiarities of the immune response in CNS. The humoral immune response in CNS differs from the immune response seen in blood. Importantly, in the CNS, there is no switch from an IgM class response to a more specific IgG class response in the course of inflammatory neurologic disease.[29,30] The pattern

of intrathecal IgG/IgA/IgM synthesis remains rather constant and depends on the cause, pathophysiology, and localization of the disease process.

Lack of an IgM to IgG switch and slow normalization of intrathecal antibody synthesis is regarded as consequences of the specific regulation of the intrathecal immune response.[31,32] From a diagnostic point of view, the lack of an IgM to IgG switch in CNS enables one to characterize disease-related patterns instead of acuity-related patterns. In the special environment of the CNS, there are other peculiarities in the immune response.

In addition to the absence of a switch from IgM to IgG that is widely used for diagnostic purposes,[30,31] regulation of the immune response shows other differences. One fact to be taken into account is that existence of immunoglobulins intrathecally synthesized does not preclude the possibility that some of these CNS immunoglobulins may have reached the CSF from blood. It is possible to differentiate the fraction of the brain-derived local synthesis by using a Reibergram.[29]

In eosinophilic meningitis caused by *A. cantonensis*, CNS damage caused by the motile worm, inflammatory responses to foreign antigens, and possible toxicity of worm components work in concert to produce the pathology and clinical disease picture. The immune response in this disease, as detected in the CSF, clearly shows these pathologic changes and clinical findings.

The classical and lectin pathways of complement activation can lead to the inflammatory process during meningitis. C4 can play an essential role by interaction with mannose-binding lectin or C2 from the classical pathway.[33] Neurotoxins produced by eosinophils during the inflammation process, including antibody production, should be the primary causes of CNS damage. C4 with immunoglobulin intrathecal synthesis occurs at least by the classical pathway. Antibody-dependent complement cytotoxicity may then occur. This type of cytotoxicity, rather than phagocytosis,[32] may eliminate *A. cantonensis* larvae.

Patients with angiostrongyliasis can be expected to manifest synthesis of C3c as a degradation product of C3. First, this expectation indicates that complement activation has occurred by at least one of the three pathways and that the involved protein was biologically used. Second, when the role of C3 is completed, it was modified into C3c in the CSF. Studies have indicated that C3 is almost exclusively responsible for facilitating the stable attachment of eosinophils and other leukocytes to the specific pathogen.[34]

The practical use of C3c as a split fraction of C3 intrathecal synthesis is a measure of the functionality of this fraction during intrathecal activation. It is also a sensitive marker of complement system activation in general. Patterns that involve C4 intrathecal synthesis always include C3c intrathecal synthesis, and provide additional information about the biological process that generally includes IgM intrathecal synthesis. All of these patterns enable a more complete picture of this important process, which can clarify the mechanism of the observed lysis of third-stage larvae of *A. cantonensis* in the CSF.

This review of the process of intrathecal inactivation during angiostrongyliasis has included those facts that we believe are most relevant. The significance of some of the observations reported is presently unknown. These observations include the role of the complement system in destruction of larvae. An understanding of this process could facilitate strategies for anti-parasitic drug design. These findings have led us to make two conclusions. First, complement is a competitive natural adjuvant, and an understanding of its manifold pathways will ultimately enable understanding of angiostrongyliasis. Second, this understanding should identify the best way to eliminate invasive larvae, even before their entry into the CNS.

Authors' addresses: Barbara Padilla-Docal, Ivonne Iglesias-González, Raisa Bu-Coifiu-Fanego, Carmen Aleida Socarrás-Hernández, and Alberto Juan Dorta-Contreras, Facultad de Ciencias Médicas Dr. Miguel Enríquez – Investigaciones, Ramón Pintó 202 Luyanó, Havana 10700, Cuba, E-mails: barbara.padilla@infomed.sld.cu, iiglesias@infomed.sld .cu, raisabu@infomed.sld.cu, carmen.hdez@infomed.sld.cu, and dorta@ infomed.sld.cu.

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