Comparison of Major Immunoglobulins Intrathecal Synthesis Patterns in Ecuadorian and Cuban Patients with Angiostrongyliasis

Bárbara Padilla-Docal, Alberto J. Dorta-Contreras, Juan M. Moreira, Luiggi Martini-Robles, Jenny Muzzio-Aroca, Fernando Alarcón, María Esther Magraner-Tarrau, and Raisa Bu-Coifí-Fanego

Abstract. Angiostrongyulus cantonensis meningitis was first reported in Cuba in 1981, and it was recently reported in South America. The aim of this paper is to evaluate the intrathecal immunoglobulin synthesis patterns from Cuba’s and Ecuador’s patients with angiostrongyliasis. Eight Ecuadorian patients from two different outbreaks and 28 Cuban patients were studied. Simultaneous blood and cerebrospinal fluid简单s were taken. Immunoglobulin (Ig) A, IgM, IgG, and albumin were quantified by radial immunodiffusion. Corresponding Reibergrams were applied. A three-Ig pattern was the most frequent in the two groups, but IgM was presented in all Ecuadorian young mature patients; however, in the Cuban children, only 12 of 28 patients had intrathecal IgM, but about 90% had an IgA and IgG synthesis at time of later puncture. This indicates that, with a larger amount of parasites ingested, clinical symptoms are more severe, and a higher frequency of intrathecal IgM synthesis could be observed. This is discussed as a similarity with the intrathecal IgM synthesis in African trypanosomiasis.

INTRODUCTION

Angiostrongyulus cantonensis is a helminth parasite. The definitive hosts of this parasite are rats; several mollusk species constitute intermediary hosts. The mature worms live in the pulmonary arteries of rats. The human is a non-permissive, accidental host. Human infections have been known to be acquired by the ingestion of the infective third-stage larvae contained in raw or inadequately cooked food—either the intermediate mollusk hosts (snails and slugs) or animals acting as paratenic host (planarians, crustaceans, frogs, monitor lizards, etc.)—and contaminated fresh vegetables, including raw vegetable juice. The term human angiostrongyliasis refers primarily to eosinophilic meningitis, the major clinical feature of A. cantonensis infection in human beings. It is considered an important and sometimes fatal human disease. The infection frequently appears in outbreaks, with a number of cases between 8 and 100 patients.

Meningitis by A. cantonensis was first described in Southeastern Asia and reported in Asia, Africa, and the Caribbean. In 1981, Cuba was the first country to report this disease in the Americas, and recently, it was reported in Ecuador. Previously, the parasite had been found in naturally infected snails of various genera and species in Northeastern Brazil, which also has reported cases of eosinophilic meningitis in the same areas.

For the most sensitive detection of a brain-derived fraction besides the blood-derived fraction of immunoglobulins, it was necessary to characterize a discrimination line in the quotient diagrams between these both fractions. Reibergrams is a valuable method to determine the intrathecal synthesis of immunoglobulins.

The humoral immune response in the central nervous system (CNS) is different from the immune response observed in blood. As a main difference, in cerebrospinal fluid (CSF), there was no switch from immunoglobulin M (IgM) class response to a more specific IgG class response in the course of inflammatory neurological diseases. The pattern of intrathecal IgG/IgA/IgM synthesis remains rather constant and depends on the cause, pathophysiology, and localization of the disease process. From a diagnostic point of view, the lack of IgM to IgG switch in CNS is the chance to characterize disease-related instead of acuteness-related patterns.

The first pattern of intrathecal Ig synthesis in a parasite-induced CNS disease was reported in eosinophilic meningoencephalitis caused by A. cantonensis. Later Reibergrams were reported in African trypanosomiasis.

The use of Reibergram or Reiber’s CSF/serum quotient diagrams in epidemiological studies has been reported previously. Different immunological patterns in CSF could be described for viral meningoencephalitis outbreaks.

The aim of this paper is to establish a comparison between Ecuadorian and Cuban patients suffering from angiostrongyliasis from the neuroimmunological point of view by using Reibergrams.

MATERIALS AND METHODS

Eight young adult patients of an average age of 23 years with a diagnosis of eosinophilic meningitis caused by A. cantonensis were studied. This study was preceded by an epidemic outbreak reported recently in Ecuador. These are the first human cases of angiostrongyliasis in humans from Ecuador. All had been eating raw snails. These patients were admitted to the Neurology Service of Eugenio Espejo Hospital of the City of Quito in December 2008 and April 2009. The research was approved by the Committee on Bioethics Research of the Hospital Eugenio Espejo. All patients gave their informed consent to carry out a diagnostic lumbar puncture and allow use of their clinical data history.

The diagnosis of Ecuadorian patients was based on the history of ingesting raw snails, clinical symptoms, and CSF test
characteristics and parasitological findings, like presence of L2
larvae in intermediate host and adult worm in lung’s finite ani-
mal species like rats. Other parasites different from *A. canton-
ensis* were excluded according to their symptoms and previous
epidemiological background and history.

The 28 Cuban patients studied were children of an average
age of 5.8 years. The patients were admitted to the Pediatric
Hospital of San Miguel Padrón of the City of Havana between
1998 and 2007 with typical symptoms. The diagnosis was based
mainly on the epidemiology, clinical features, neuroimmunological
response, and illness development, because, in Cuba, there
is no other parasite that can cause eosinophilia in the CSF.2,3

The research project was approved by the Pediatric
Hospital of San Miguel Padrón Ethical Committee, and writ-
ten informed consent of the parent or guardian was obtained.
The serum and CSF samples were obtained simultaneously.
The samples were collected at the time of admission at the
onset of the symptoms in Cuban patients, and a second pair
of samples was taken 8 days later; these samples were kept in
small aliquots at 80°C until analysis. In Ecuadorian patients,
the first samples of CSF and serum were taken only 2 weeks
after the onset of the symptoms.

**Serum and CSF analysis.** Major Ig levels in serum from
Ecuadorian and Cuban patients were quantified using a
radial immunodiffusion technique with NOR Partigen plates
(Siemens, Marburg, Germany), and in CSF, levels were
quantified using LC Partigen plates (Siemens).

To identify whether there was intrathecal synthesis of Igs
and to determine its relationship to the blood–CSF barrier
function, albumin in serum and CSF was quantified using the
same procedures with NOR and LC Albumin Partigen plates
(Siemens).

**Reibergrams.** Igs and albumin concentrations were plotted
as CSF/serum quotients in a Reiberogram or Reiber’s quotient
diagram for major Ig classes.16,17

Reibergrams are based on molecular diffusion/CSF flow
theory, and the fundamental principle is that a decrease in the
CSF flow rate is always accompanied by an increased concen-
tration of blood-derived proteins that is followed secondarily
by an increase in molecular diffusion from blood to CSF.25,26

To determine whether there is dysfunction of the blood–
CSF barrier and intrathecal synthesis of IgA, IgM, and IgG,
the Reibergrams16,17 have been used.

**RESULTS**

The main symptoms in the Ecuadorian patients appeared 2
weeks after consumption of raw snails. These symptoms were
characterized by fever, vomiting, neurological disorders, con-
stipation, nausea, abdominal pain, headache, and severe radic-
ular pain in all patients. Twelve days after the first symptoms,
two patients had worsening symptoms, adding slightly stiff
neck and alteration of consciousness. One of them fell into
coma. Then, it was decided to perform lumbar puncture on the
entire group.

The main symptoms in the Cuban children were fever,
vomiting, headache, and some meningeal signs in 50% of the
patients.

Intrathecal Ig synthesis patterns in the Ecuadorian patients
and their frequency are shown in Figure 1. A three-class
response was the most frequent, with five of eight cases. All
eight of eight cases showed an intrathecal IgM response.

![Reibergrams of Ecuadorian patients suffering from *A. cantonensis* meningitis. The upper hyperbolic curves (bold lines) represent the discrimination lines between brain-derived and blood-derived Ig fractions. Values above these upper discrimination lines represent intrathecal IgG, IgA, or IgM synthesis. The dashed lines indicate the extent of intrathecal synthesis as intrathecal fractions (IgGIF, IgAIF, or IgMIF) with 20%, 40%, 60%, and 80% of the measured total Ig concentration in CSF, with reference to the discrimination line as 0% intrathecal synthesis. The limit of the reference range for QAlb between normal and increased CSF protein concentrations because of blood–CSF barrier dysfunction is indicated by the age-dependent vertical lines at QAlb 5 × 10⁻³ (up to 15 years), QAlb 6.5 × 10⁻³ (up to 40 years), and QAlb 8 × 10⁻³ (up to 60 years). Values below the lower hyperbolic line in range 5 indicate a methodological fault.14,15](image-url)
There was no intrathecal Ig synthesis at time of the onset of the symptoms in the Cuban patients according to the first early diagnostic lumbar puncture. The frequencies in the second lumbar puncture are shown in Figure 2. A three-class immune response was the most frequent in 20 of 28 cases. However, 8 of 28 cases did not develop an intrathecal IgM synthesis at this time, about 8 days after start of the symptoms.

Ig intrathecal synthesis in the Ecuadorian patients is shown in Figure 1. Three patients had a blood–CSF barrier dysfunction with a Q albumin value greater than $7 \times 10^{-3}$. This situation corresponds with the time of sampling, which does not coincide with the acute face of disease. Five of eight patients show a normal blood–CSF barrier function and intrathecal synthesis of IgG + IgA, but all of them synthesized IgM. It is easy to notice, because their respective CSF/serum quotients were placed over the hyperbolic bold line in the IgM Reibergram.

In the first lumbar puncture, the Cuban patients showed a blood–CSF barrier dysfunction without an intrathecal IgG, IgA, and IgM synthesis, but in the second lumbar puncture, some of the Cuban patients (Figure 2) still showed a blood–CSF barrier dysfunction. In the second lumbar puncture, the Ecuadorian patients showed a blood–CSF barrier dysfunction. Samples from these Ecuadorian were not taken in the acute phase when the first symptoms started.

The most interesting result was that IgM presented in all of the Ecuadorian patients’ patterns compared with the Cubans’ patterns, where IgG was the major Ig always present.

DISCUSSION

Helminth *A. cantonensis* transmission has never been reported in Ecuador until the middle of 2008 when a first outbreak occurred. In December 2008, a second outbreak was reported in the province of Santo Domingo de los Tsachilas. The patients included in this paper were part of two outbreaks localized in Guayas and Los Ríos that occurred in March and April 2009.

At the sites where the patients live, L3 larvae were observed in snails, and mature worms were observed in lungs of dissected rats.

At the beginning, health authorities denied that a species used for human consumption was involved, because only wild-caught snails were infected. Probably some infected imported snails or introduced rats produced the outbreaks, but one does not know exactly how it came into the country. It is most likely that infected snails were imported by the domestic industry. When improperly handled on farms, they were accessible to wild rats, which became infected for the first time.

The sources of the contamination were a yard near Santo Domingo, where the patients collected snails, which they ate raw with lemon and salt, and a store that sells snails supplied by the domestic industry. Some snails from these sources that remained at patients’ home were contaminated with *A. cantonensis* larva. Life cycles were completed at the Parasitology Laboratory of the Instituto Nacional de Higiene Dr. Leopoldo Izquieta Perez, Guayaquil, Ecuador. In several regions from this country, one or two snail species constitute intermediate hosts, *Pomacea lineada* and *Pomacea* sp., and their infestation intensity is very high.

The first report of *A. cantonensis* in Cuba was published in 1981, but there is some unprinted evidence that the disease appeared some years before.
At the beginning, it was not easy to realize that a parasite that was reported in Southeastern Asia was settled in the New World. This parasite is endemic in Cuba and in the Caribbean basin, and the spread of the disease is by accidental uptake of larvae by children without proper hygienic habits like washing hands after contact with contaminated vegetables, soil, and snails. There is no tradition to eat raw snails in Cuba.27

The patients in this study came from the Municipality of San Miguel Padrón in the City of Havana. This consists of 150,000 inhabitants, is on the periphery of the Cuban capital, and has rural and semi-rural areas. All of the affected children lived in houses with earth patios, where it is common to also find terrestrial snails and rats, which are, respectively, the intermediate and finite hosts of the parasite.27,28

From 1981, the Pediatric Hospital San Miguel received all of the patients from the municipality—a total of 90 patients corresponding to an incidence of two to three cases per year.

Some of the Ecuadorian patients have neurological sequelae, but in the Cuban patients, no neurological sequelae were reported, probably because of the accidental intake and therefore, low quantity of swallowed larvae.26,27

Because the CSF and serum samples from the Ecuadorian patients were not taken at the beginning of the symptoms, only 37% of patients had a blood–CSF barrier dysfunction. These results are similar with the Cuban findings for the second lumbar puncture taken 8 days after the initial diagnostic lumbar puncture, where only 21.4% of Cuban patients showed a blood–CSF barrier dysfunction compared with almost 100% in the initial phase of the disease.

These findings are interesting, because the Cuban patients are children and Ecuadorians are young adults. The median albumin quotient of the children group at 8 days after start of symptoms is QA Alb = 3.8. In the Ecuadorian group, the median QA Alb = 6.5. This difference reflects mainly the age-related decrease of CSF flow rate with increasing age (i.e., increase of QA Alb).27,28

It is a remarkable finding that the intrathecal synthesis pattern of major Igs varies in Ecuadorian and Cuban patients. IgM is always present in the different patterns in the people from Ecuador suffering from this parasite neuroinfection. This typical feature is similar to the reported cases of African trypanosomiasis with involvement of the brain (stage II and III) that used Reibergrams also.26,29

In African trypanosomiasis, IgM is associated with a special type of B cell, called Mott cell, and it can be observed in other neuroinflammatory diseases like neurosyphilis.30

A predominant IgM response is also observed in Lyme neuroborreliosis and mumps meningoencephalitis. Isolated IgM synthesis is occasionally observed in non-Hodgkin's lymphoma involving the CNS.18 These examples, however, indicate that the Ig pattern can guide the choice of further analyses, such as detection of specific antibody synthesis, use of polymerase chain reaction, and detection of the causative microorganism.26,29,30

IgM and interleukin-10 (IL-10) in CSF are typical components of the neuroinflammatory response in patients with the brain stage of Trypanosoma brucei gambiense infection.31

The intrathecal synthesis patterns in Cuban adults do not vary compared with children, and it is a typical immune response that supports the diagnosis. IgG is always present in such patterns.19,21,22

Increment of intrathecal IgM synthesis may have a connection with the quantity of the larvae intake. Additionally, the differentiation in the neuroimmunological patterns found in Ecuadorians and Cubans may be explained by taking into account additional factors like age and sampling time.

In both groups, a three-class intrathecal synthesis pattern is the most frequent pattern. This IgG + IgA + IgM pattern is reported to be related with the special pathological mechanisms of the different diseases, the time of sampling, and the brain localization of the infection.19,22

Differentiation in the neuroimmunological patterns found in Ecuadorians and Cubans may be explained by taking into account the parasite–host interaction, survival strategies of the parasite in the environment, defense mechanisms of human hosts, like complement activation,19,20 and quantity of larvae intake.

CONCLUSIONS

The CSF data pattern in angiostrongylus infection depends on time of puncture in the course of the disease, age of the patients, and particularly, amount of parasites ingested. An immediate appearance of the blood–CSF barrier dysfunction is followed by an intrathecal immune response 1–2 weeks after start of clinical symptoms. Besides a frequent three-class immune response, the intrathecal IgM response seems to be more frequent with higher amounts of parasite ingested. This is a new aspect that adds to the earlier observation that, in African trypanosomiasis, the appearance of intrathecal IgM synthesis is the most sensitive diagnostic sign for involvement of the brain.

Received September 8, 2010. Accepted for publication December 1, 2010.

Acknowledgments: The authors would like to thank Manuel Rodriguez for technical assistance and the reviewers for their helpful comments. The American Society of Tropical Medicine and Hygiene (ASTMH) assisted with publication expenses.

Authors’ addresses: Bárbara Padilla-Docal, Alberto J. Dorta-Contreras, María Esther Magraner-Tarrau, and Raísa Bu-Coifiu-Fanego, Laboratorio Central de Líquido Cefalorraquiédico (LABCEL), Facultad de Ciencias Médicas Dr. Miguel Enríquez, Universidad de Ciencias Médicas de La Habana, Cuba, E-mails: barbara.padilla@infomed.sld.cu, adorta@infomed.sld.cu, mariae.magraner@infomed.sld.cu, and raisabu@infomed.sld.cu. Juan M. Moreira, Dirección de Control y Mejoramiento de la Salud Pública, Ministerio de Salud Pública, Quito, Ecuador, E-mail: jmoreira@msp.gov.ec. Luiggi Martini-Robles and Jenny Muzzio-Aroca, Departamento de Parasitología, Instituto Nacional de Higiene, Dr. Leopoldo Izquieta Perez, Guayaquil, Ecuador, E-mails: luiggi.martinir@hotmai.com and jennymuzzio@yahoo.com. Fernando Alarcón, Servicio de Neurología, Hospital Eugenio Espejo, Quito, Ecuador, E-mail: falarc2000@hotmail.com.

REFERENCES


12. Reiber H, 1994. Flow rate of cerebrospinal fluid (CSF): a concept common to normal blood–CSF barrier function and to dysfunc-


15. Dorta-Contreras AJ, 2001. Reibergrama como herramienta epide-

16. Dorta-Contreras AJ, 2006. Pattern of intrathecal immunoglobulin synthesis in pediatric patients with infectious meningocere-


