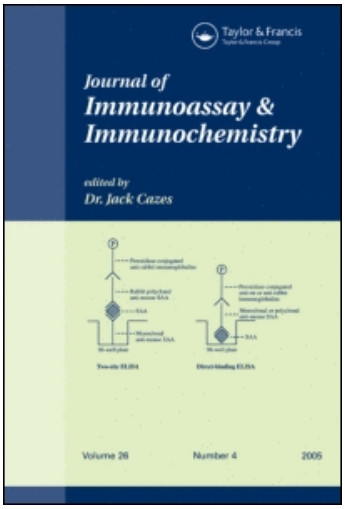

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

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Muralidhar K. Katti[a]; Myna T. Acharya[a]
[a] Immunology Laboratory, Department of Microbiology, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, India

Online publication date: 30 November 2001

To cite this Article Katti, Muralidhar K. and Acharya, Myna T.(2001) 'IMMUNODIAGNOSIS OF TUBERCULOUS MENINGITIS: DETECTION OF ANTIBODY REACTIVITY TO ANTIGENS OF *MYCOBACTERIUM TUBERCULOSIS* AND *CYSTICERCUS CELLULOSAE* IN CEREBROSPINAL FLUID TUBERCULOUS MENINGITIS PATIENTS BY ELISA', Journal of Immunoassay and Immunochemistry, 22: 4, 401 — 406
To link to this Article: DOI: 10.1081/IAS-100107403
URL: http://dx.doi.org/10.1081/IAS-100107403

J. IMMUNOASSAY & IMMUNOCHEMISTRY, 22(4), 401–406 (2001)

IMMUNODIAGNOSIS OF TUBERCULOUS MENINGITIS: DETECTION OF ANTIBODY REACTIVITY TO ANTIGENS OF *MYCOBACTERIUM TUBERCULOSIS* AND *CYSTICERCUS CELLULOSAE* IN CEREBROSPINAL FLUID TUBERCULOUS MENINGITIS PATIENTS BY ELISA

Muralidhar K. Katti* and Myna T. Acharya

Immunology Laboratory, Department of Microbiology,
Sree Chitra Tirunal Institute for Medical Sciences and
Technology, Thiruvananthapuram 695 011, India

ABSTRACT

Enzyme-linked immunosorbent assay (ELISA) was standardized and evaluated for detection of antibody response in cerebrospinal fluid (CSF) to antigens of *Mycobacterium tuberculosis* and *Cysticercus cellulosae*. Sonicated extracts of heat killed *M.tuberculosis* H37Rv and *C.cellulosae* were prepared and used in ELISA to detect respective antibody response in CSFs for a definitive diagnosis as to tuberculous meningitis

* Corresponding author. E-mail: mkk@sctimst.ker.nic.in

(TBM)/neurocysticercosis (NCC). ELISA was performed in a total of 201 CSF samples, which include Group I: chronic infections of the central nervous system (CNS) with possible diagnosis of TBM, tuberculoma, or NCC ($n = 70$), and Group II: control group of patients with infectious neurological ($n = 19$), non-infectious neurological ($n = 82$), and non-infectious non-neurological conditions, i.e., spinal anaesthesia CSFs ($n = 30$). Specificity in this study was 99.9% and no true cross-reactivity between antimycobacterial antibodies and *C.cellulosae* antigens and vice-versa was observed. However, in 17.14% of CSFs (12/70), both antimycobacterial and anticysticercal antibodies were detected, 50% of these cases were diagnosed as TBM. But none of the proven NCC cases showed presence of antimycobacterial antibodies. Results of this study would indicate that it would be beneficial if both antibody and antigen responses are detected in CSFs to infectious aetiologies such as *M.tuberculosis*, *C.cellulosae*, and *C.neoformans* in order to enhance the diagnostic accuracy and proper management, as these diseases are highly endemic in underdeveloped and developing countries.

INTRODUCTION

Tuberculous meningitis (TBM) is one of the most common chronic infections of the central nervous system (CNS).(1) Other causes of chronic infection of the CNS which are prevalent in underdeveloped and developed countries of the Indian subcontinent are bacterial (neurobrucellosis, neurosyphilis), parasitic (neurocysticercosis NCC, toxoplasmosis), fungal (cryptococcal meningitis), or viral (HIV-1 and HTLV-III).(2) The diagnosis of TBM has been problematic, as it causes various clinical manifestations that can be confused with those of other chronic infections of the CNS such as NCC, cryptococcal, or carcinomatous meningitis.(2) Definitive diagnosis among TBM, NCC, or cryptococcal meningitis is essential because treatment for the one is contraindicated for the other.(3)

Tuberculous aetiology is confirmed by the demonstration of acid-fast bacilli (AFB), either in smear or culture. These methods are insensitive (Zeil-Nelson staining) and time consuming (culture technique) with the culture yield rate of as low as $< 1\%$ at our centre. Therefore, detection of indirect evidence like specific antibody/circulating antigen resulting as a consequence of hosts' immune response are of paramount importance in aetiological confirmation of clinical diagnosis.

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We present, here, an interesting observation of the importance of detection of antibody response to antigens of *Mycobacterium tuberculosis* and *Cysticercus cellulosae* in CSFs of certain TBM cases.

EXPERIMENTAL

Antigens

Mycobacterium tuberculosis Sonicate Extract (MTSE)

MTSE was prepared as described elsewhere.(4) Briefly, *M. tuberculosis* H37Rv (obtained from the National Tuberculosis Institute, Bangalore, India) was grown on Loweinstein-Jensen medium to its log phase (3–4 weeks) at 37°C. The culture was harvested in phosphate buffered saline (PBS, pH 7.2), heat killed at 60°C for 1h, then subjected to ultrasonication in an ice bath. The sonicate was centrifuged at high speed (17,000 g) and the supernatant was estimated for protein content by the Bradford method(5) and stored frozen in aliquots until use.

Porcine *Cysticercus cellulosae* Sonicate Extract (PCSE)

PCSE was prepared according to Katti and Chandramuki.(6) Non-ruptured, carefully dissected, whole cysts from infested pork were washed, homogenized in PBS, pH 7.2, ultrasonicated, and centrifuged at 17,000 g. The protein content of the supernatant was estimated(5) and stored frozen in aliquots.

Hyper Immune Sera

Rabbit antisera were raised against MTSE and PCSE by injecting rabbits intradermally with 1.5 mg of one antigen each, bleeding the animals 8 to 10 days later, and purifying IgG from sera.

CSF

CSFs from 201 patients were included from the following group of patients: Group I: chronic infections ($n = 70$) based on clinical findings plus one or more of the following criteria (i) CSF parameters (pleocytosis,

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elevated protein level, and low glucose level); (ii) neuroimaging – showing hydrocephalus, basilar enhancement; and (iii) evidence of concomitant extra neurological tuberculous localization. Group II: controls ($n = 131$) including (a) non-infectious non-neurological condition (CSF collected during spinal anaesthesia) ($n = 30$); (b) infectious neurological conditions (culture proven bacterial and serologically confirmed viral meningitis) ($n = 19$); and (c) non-infectious non-neurological conditions (such as disc prolapse, carotid insufficiency brain tumors etc) ($n = 82$).

ELISA

The ELISA was performed according to the method described elsewhere.(6) MTSE and PCSE antigen solutions in PBS, pH 7.2, were coated onto polystyrene plates (Immunolon I Dynatech) at 4°C overnight. Five-fold dilutions of each CSF in PBS-diluent (2% skimmed milk powder in PBS, pH7.2) with an initial dilution of 1 : 5 were tested. A 1 : 1000 dilution of horse radish peroxidase (HRPO)-antihuman IgG (Dakopatts) was used with o-phenylene diamine hydrochloride (OPD, Sigma) in phosphate citrate buffer of pH 5.0 with 0.012% H_2O_2 as substrate. The plate was read at 492 nm.

RESULTS AND DISCUSSION

In view of the problems associated with definitive diagnosis as to TBM/NCC, ELISA was standardized and evaluated using MTSE and PCSE antigens and their respective rabbit hyper immune antibodies. The results of ELISA using MTSE and PCSE are shown in Table 1. ELISA in this study determined to be specific as the control group of CSFs did not show any reactions to either MTSE or PCSE, nor was there any true cross-reactivity between anti mycobacterial antibody and PCSE or vice-versa by immunoblot assay.(7) Hence, our results (Table 1) indicate that 11.42% and 12.85% of CSFs are true positives for MTSE and PCSE, respectively. However, in over 17% of CSFs, both anti mycobacterial and anti cysticercal antibodies were detected. On an individual analysis of titers of those CSFs which reacted to both MTSE and PCSE (Table 2), six (50%) of them were diagnosed as TBM, 3 (25%) as GBS, and 2 remained as chronic meningitis.

We are unsure whether this type of non-specific reactivity can occur between proteins derived from prokaryote and eukaryote. It is interesting to note that only certain cases of TBM had cysticercal antibodies, while

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Table 1. Detection of Antibody Responses to MTSE & PCSE in CSF Samples of Patients Chronic Infections of the CNS and Controls by ELISA

Group	Number Tested	Number of Positive Response*		
		MTSE	PCSE	MTSE & PCSE
Chronic infections control	70	08 (11.42%)	09 (12.85%)	12 (17.14%)
Non infectious non neurological conditions	30	0	0	0
Infectious neurological conditions	19	01	0	0
Non infectious neurological conditions	82	0	0	0

*Dilution of $\geq 1:5$ was considered as positive at the OD_{492} values of 0.127 for MTSE and 0.085 for PCSE.

anti mycobacterial antibodies were not found in proven cases of NCC. Even by a passive haemagglutination assay system, a similar type of antibody reactivity was also observed (data not shown). Immunoblot analysis of these CSFs showed antibody reactivity to 70-kDa and 10-kDa antigens of *C. cellulosae* (data not shown). Transudation or exudation of parasitic antibodies from systemic circulation to the CNS compartment through an inflamed blood-brain-barrier(8) and induction of an immune response to stress proteins of *M. tuberculosis* which may have sequence homology with that of eukaryotic proteins resulting in positive reactions,(9) would possibly explain the presence of anticysticercal antibodies in certain cases of TBM. Therefore, we emphasize that it is essential to look for antibody response to both MTSE and PCSE in achieving differential diagnosis. Nevertheless, detection of circulating antigen(s) of *M. tuberculosis*, *C. cellulosae* and *C. neoformans*, per se, in CSFs would be more advantageous to diagnose differentially as to TBM/NCC/cryptococcal meningitis, especially where these diseases are endemic.

ACKNOWLEDGMENTS

The Department of Science and Technology, Kerala State Government, is gratefully acknowledged for financial support. We express our thanks to the Director, SCTIMST, Trivandrum, for allowing us to carry out this study. MTA was a short-term Fellow of the Indian Science Academy.

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Received June 18, 2001 Manuscript 3043
Accepted July 24, 2001

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