

Analysis of Tuberculous Meningitis Cases by an Immunoblotting Assay Based on a Mycobacterial Antigen Complex

Y. L. ZOU,¹ M. P. VAN ANTWERPEN,² G. Q. SHI,³ Q. X. CHEN,³ C. J. M. SINDIC,² AND C. COCITO^{1*}

Microbiology & Genetics Unit, ICP, University of Louvain,¹ and Laboratory of Neurochemistry, St. Luc University Clinics,² Brussels, Belgium, and Tuberculosis Hospital, Chao Yang, People's Republic of China³

Received 10 November 1993/Returned for modification 10 January 1994/Accepted 17 February 1994

Tuberculous meningitis cases were analyzed by an immunoblotting test based on *Mycobacterium bovis* BCG antigen complex A60. Anti-A60 immunoglobulin G (IgG) in cerebrospinal fluid (CSF) allowed early diagnosis, and concentrations decreased after recovery. In primary meningitis forms, anti-A60 IgGs were intrathecally synthesized and specific oligoclonal IgGs were present in CSF. In meningeal complications of pulmonary tuberculosis, there were matching titers of anti-A60 IgG in blood and CSF (mirror pattern). Correlation between CSF-restricted patterns and CSF pleocytosis was shown.

A recent increase in tuberculosis incidence and complications has been registered in connection with the spread of antibiotic resistance and AIDS. Tuberculous meningitis is a complication invariably lethal without early therapy (10, 33); however, identification of *Mycobacterium tuberculosis* in the cerebrospinal fluid (CSF) is a rare event, and in vitro culture is a lengthy process. Detection of PCR-amplified mycobacterial DNA by nucleic acid probes (32, 40) and of antimycobacterial immunoglobulins in CSF by radioimmunoassays (30), enzymometric methods (enzyme-linked immunosorbent assay) (1, 6, 7, 15-17, 25-27, 29, 31, 34, 38, 39, 44, 46), and latex agglutination (36) have been explored insufficiently at the clinical level. Literature in this field has been extensively reviewed (8, 12, 22, 35, 45).

The thermostable macromolecular antigen complexes from mycobacteria (12, 20) (A60 of *Mycobacterium bovis* and A36 of *Mycobacterium paratuberculosis*) elicit humoral and cellular immune reactions (3, 13) and are immunodominant in mycobacterioses (12). A60- and A36-based immunoassays have been applied to diagnosis of tuberculosis (2, 9, 12, 21, 23, 24) and paratuberculosis (18), respectively. We have previously explored the use of immunoassays in meningitis (4, 5) and described an A60-based immunoblotting procedure for analysis of the CSF of tuberculous meningitis patients (41). By this method, a very large proportion of antimycobacterial immunoglobulin G (IgG) in patients' CSF was shown to be directed against A60 (41). This study is complemented by the present work, in which samples of blood and CSF from patients with proven or suspected tuberculous meningitis were subjected to A60 immunoblotting analysis at different phases of the disease.

Our study was carried out on 80 CSF samples from 32 Chinese patients (19 females and 13 males) of various ages (between 5 months and 66 years): 15 patients with proven tuberculous meningitis (positive Ziehl-Neelsen staining and/or CSF culture) and 17 patients with suspected tuberculous meningitis (negative CSF culture). Some patients (25 of 32) had radiographically confirmed pulmonary tuberculosis with open lesions (18 of 25 exhibited sputum positivity). Only one individual had been vaccinated with *M. bovis* BCG and was positive in purified protein derivative cutaneous testing. Sub-

jected to standard chemotherapy, two patients died, whereas the others recovered either completely (20 of 32) or partly (10 of 32) (residual sequelae and/or impairment of blood-CSF barrier). Standard CSF analyses (cell number and glucose) were performed periodically. A control group included 2 healthy persons and 15 nontuberculous patients (1 leukemia, 1 lupus erythematosus, 1 Guillain-Barré syndrome, 1 cerebral cysticercosis, 4 meningococcal meningitis, 2 viral meningitis, 2 hypertensive headache, 2 ischemic stroke, 1 cerebral tumor). Cases of uncomplicated miliary tuberculosis or miliary tuberculosis complicated with tuberculous meningitis were included in our study.

At different times (up to 14 years after the clinical onset), paired samples of CSF and serum were collected and kept frozen at -20°C. Protein content was determined by a nephelometric micromethod using benzethonium chloride in microtiter plate wells (37), and IgG levels were determined by immunoturbidimetry (Behring, Marburg, Germany). Serum samples were diluted with H₂O to the same IgG concentration as the paired CSF samples. Aliquots (10 µl) of CSF and diluted sera were applied side by side on agarose gel plates (pH gradient, 3.5 to 9.5), which were prepared in distilled water with 0.36 g of Agarose-IEF, 2 ml of Ampholine (Pharmacia, Uppsala, Sweden), and 4.3 g of sorbitol (Merck, Darmstadt, Germany). Samples were isoelectrically focused for 70 min at 10°C in an electrophoretic unit (Multiphor; LKB, Uppsala, Sweden).

Gels were blotted (40 min, 10°C) onto A60-loaded polyvinylidene difluoride (PVDF) membranes (Immobilon; Millipore, Bedford, Mass.) under a uniform weight of 1 kg. A60-PVDF membranes were prepared as follows. PVDF membranes were successively steeped in methanol and water, incubated overnight with A60 (100 µg/ml), and rinsed three times for 20 min with TBST buffer (500 mM NaCl, 20 mM Tris-HCl [pH 7.5] containing 0.05% Tween 20). A60 prepared from the cytoplasm of *M. bovis* BCG (Pasteur Institute, Brussels, Belgium) by exclusion gel chromatography on Sepharose 6B columns (Pharmacia), as described previously (14), was identified by two-dimensional immunoelectrophoresis according to an established reference system (11) and quantitated by spectrophotometric measurement of its protein content (20). Immunoblots were washed with TBST buffer and fixed with glutaraldehyde (0.25% solution in TBST buffer, 20 min, 4°C) (28). After repeated washings with buffer, membranes were incubated with alkaline phosphatase-labeled anti-

* Corresponding author. Mailing address: Microbiology and Genetics Unit, University of Louvain, GEMO, VESALE, UCL 5225, Avenue Mounier 52, B-1200 Brussels, Belgium. Phone: 2/764.52.25. Fax: 2/764.53.22.

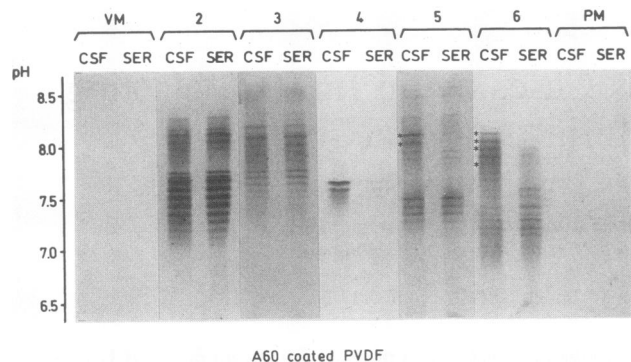


FIG. 1. A60 immunoblotting analysis of CSF and sera from meningitis patients. Immunoblots of A60-specific IgG from paired samples of CSF and serum (SER), diluted to the same IgG concentration, after isoelectric focusing in agarose gel and immunoaffinity transfer to PVDF sheets coated with A60. Two samples from patients with purulent meningitis (PM) and viral meningitis (VM) were used as controls. In lanes 2 (tuberculous meningitis complicated by postprimary tuberculosis) and 3 (tuberculous meningitis complicating miliary tuberculosis), A60-specific IgG oligoclonal bands of similar intensities were stained in CSF and serum (mirror pattern). In lanes 4 and 6 (tuberculous meningitis alone) and lane 5 (tuberculous meningitis complicating miliary tuberculosis), A60-specific CSF-restricted oligoclonal IgG indicated an intrathecal synthesis.

human IgG (Bio-Rad, Richmond, Calif.) (1/1,500 dilution in TBST buffer containing 0.33% defatted milk, 60-min incubation at room temperature), followed by three 5-min washings with buffer and staining with the alkaline phosphatase substrate 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium (Bio-Rad).

Samples from controls (healthy persons and persons with

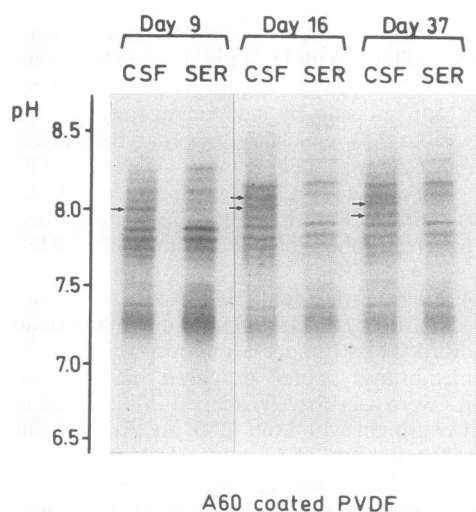


FIG. 2. A60 immunoblots of CSF and serum samples taken from a patient at different stages of disease. Immunoblots of A60-specific IgG from paired samples of CSF and serum (SER), diluted to the same IgG concentration, after isoelectric focusing in agarose gel and transfer by immunoaffinity to PVDF sheets coated with A60. Paired samples were collected, at days 9, 16, and 37 after clinical onset, from a patient with tuberculous meningitis complicating miliary tuberculosis. The total amounts of IgG in gels were 19.8, 6.5, and 7.3 μ g, respectively. The greater intensity of staining of some oligoclonal IgG antibodies in the CSF indicated an intrathecal synthesis (arrows).

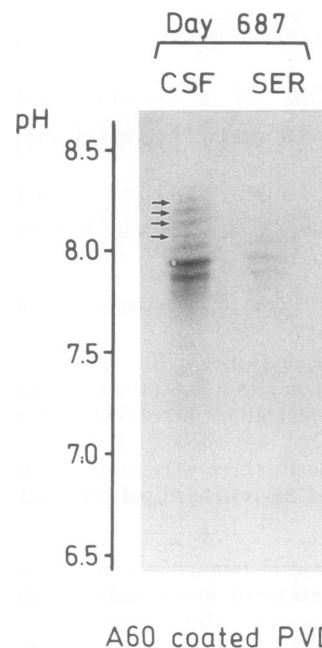


FIG. 3. A60 immunoblots of CSF and serum samples taken from a patient about 2 years after onset of disease. When admitted to the hospital, the patient presented with severe meningitis and, after recovery, permanent sequelae. The greater intensity of staining of some oligoclonal IgG antibodies in the CSF and the characteristic CSF restriction of these bands (arrows) indicated an intrathecal synthesis of antimycobacterial immunoglobulins.

nontuberculous neurological diseases) did not contain anti-A60 IgG. Immunoblots from all tuberculous meningitis patients invariably bore stained bands of anti-mycobacterial IgG. In most cases of tuberculous meningitis complicating miliary and postprimary pulmonary tuberculosis, the intensities of stained bands in paired CSF and serum samples (taken from the same individual at the same time) were comparable (mirror patterns in Fig. 1). However, in some patients (primary tuberculous meningitis without severe lung complications), the intensity of stained bands in CSF was higher than that of the paired serum sample, thus indicating an intrathecal synthesis of specific immunoglobulins. In the latter case, CSF-restricted oligoclonal bands (Fig. 2, arrows) were also present, an additional proof of *in situ* antimycobacterial IgG formation. Similar observations, in cases of viral meningoencephalitis, have been related previously (19, 42, 43). Samples taken with increasing frequency from the same subject showed A60-specific IgG bands of increasing intensities, which disappeared, in parallel with CSF pleocytosis, after recovery, in agreement with previous reports (39, 41). However, in a few instances (severe meningitis with permanent sequelae), high levels of antimycobacterial IgG were still present 2 years after disease onset (Fig. 3).

Analyzed cases of tuberculous meningitis were classified in four groups, one for primary meningeal forms and the others for meningeal complications of primary and postprimary pulmonary and miliary tuberculosis (Table 1). Patients with proven intrathecal synthesis of anti-A60 IgG (Fig. 1, lanes 4 to 6) were unevenly distributed among the four groups; they were few in the case of meningitis complicating pulmonary tuberculosis and numerous in primary meningitis and meningeal complications of miliary tuberculosis (Table 1). We found a correlation between anti-A60 IgG levels in CSF and blood and

TABLE 1. Clinical data and anti-A60 antibody production in CSF of tuberculous meningitis patients

Groups (no. of cases)	Microbiological analysis ^a		PPD + skin test (no. positive) ^b	No. (%) showing intrathecal synthesis of anti-A60 antibodies	No. showing mirror pattern
	CSF +	Sputum +			
Tuberculous meningitis alone (7)				5 (71)	2
5 bacteriologically proven	5	0	5		
2 clinically suspected	0	0	2		
Primary pulmonary tuberculosis + tuberculous meningitis (3)				1 (33)	2
1 bacteriologically proven	1	1	1		
2 clinically suspected	0	2	2		
Miliary tuberculosis + tuberculous meningitis (18)				8 (44)	10
8 bacteriologically proven	8	5	6		
10 clinically suspected	0	7	8		
Postprimary pulmonary tuberculosis + tuberculous meningitis (4)				0 (0)	4
1 bacteriologically proven	1	1	1		
3 clinically suspected	0	2	3		

^a Number of cases positive. Microbiological positivity in CSF was obtained by culture. Some of the culture-positive sputum samples were also identified by staining.

^b PPD, purified protein derivative.

CSF pleocytosis (Table 2). Most samples (18 of 21) with normal cell numbers in CSF had similar anti-A60 IgG titers in blood and serum (mirror pattern), whereas in samples with pleocytosis 27 of 56 displayed CSF-restricted anti-A60 IgG (intrathecal synthesis) ($P < 0.01$). In this connection, it ought to be mentioned that, while all of the analyzed adult cases of primary meningitis presented with intrathecal synthesis of antimycobacterial antibodies, newborns (Table 1, first row) displayed mirror patterns.

Since mycobacterial staining in CSF is invariably negative and culture positivity can be demonstrated, after long delays, in only a minority of cases, early diagnosis of tuberculous meningitis (which is essential for recovery) ultimately relies on sensitive and fast molecular biology techniques. Hybridization of labeled probes with segments of the mycobacterial genome, duly amplified by PCR, is presently investigated in several laboratories, including ours. An alternative procedure is the serological identification, in the CSF, of antimycobacterial immunoglobulins. The handicap of the PCR probe approach is the expansion of contaminating signals. The immunoblot technique, which exploits the clonal expansion of specific B lymphocytes, is less prone to contamination problems. In addition, appearance and disappearance of specific oligoclonal IgG species during the course of the disease may provide precious

information about the efficiency of chemotherapeutic intervention.

The following conclusions can be drawn from our data. (i) Anti-A60 CSF IgG is an early symptom of tuberculous meningitis. (ii) Anti-A60 IgG titer decreases in parallel with pleocytosis upon successful therapy. (iii) Matching anti-A60 IgGs in blood and serum (mirror pattern) are common in cases of meningeal complications of pulmonary tuberculosis. (iv) CSF-restricted anti-A60 IgGs (intrathecal synthesis) are specific to primary tuberculous meningitis and meningeal complications of miliary tuberculosis.

REFERENCES

- Ashtekar, M. D., A. S. Dhalla, T. B. M. S. Mazarelo, and A. M. Samuel. 1987. A study of *Mycobacterium tuberculosis* antigen and antibody in cerebrospinal fluid and blood in tuberculous meningitis. Clin. Immunol. Immunopathol. 45:29-34.
- Baelden, M. C., B. Vanderelst, M. Dieng, J. Prignot, and C. Cocito. 1990. Serological analysis of human tuberculosis by an ELISA with mycobacterial antigen 60. Scand. J. Infect. Dis. 22:63-73.
- Benoit, C., A. Beschin, M. Desmecht, P. Dekeyser, and C. Cocito. 1989. Delayed hypersensitivity reactions by the mycobacterial antigen A60 and cutaneous testing in tuberculosis. Med. Microbiol. Immunol. 178:105-112.
- Boucquey, D., M. P. Chalon, C. J. M. Sindic, M. E. Lamy, and C. Laterre. 1990. Herpes simplex virus type 2 meningitis without genital lesions: an immunoblot study. J. Neurol. 237:285-289.
- Bukasa, K. S. S., C. J. M. Sindic, M. Bodeus, G. Burtonboy, C. Laterre, and J. Sonnet. 1988. Anti-HIV antibodies in the CSF of AIDS patients: a serological and immunoblotting study. J. Neurol. Neurosurg. Psychiatry 51:1063-1068.
- Chandramuki, A., P. R. J. Allen, M. Keen, and J. Ivanyi. 1985. Detection of mycobacterial antigen and antibodies in the cerebrospinal fluid of patients with tuberculous meningitis. J. Med. Microbiol. 20:239-247.
- Chandramuki, A., G. H. Bothamley, P. J. Brennan, and J. Ivanyi. 1989. Levels of antibody to defined antigens of *Mycobacterium tuberculosis* in tuberculous meningitis. J. Clin. Microbiol. 27:821-825.
- Chaparas, S. D. 1982. The immunology of mycobacterial infection. Crit. Rev. Microbiol. 9:139-197.
- Charpin, D., H. Herbault, M. J. Gevaudan, M. Saadjian, P. De Micco, A. Arnaud, et al. 1990. Value of ELISA using A60 antigen

TABLE 2. Correlation between CSF pleocytosis and intrathecal synthesis of anti-A60 antibodies^a

No. of cells in CSF (cells/ μ l) ^b	No. of samples with the following immunoblotting pattern:		Total no.
	Mirror pattern	CSF-restricted pattern	
≤ 8	18	3	21
> 8	29	27	56
Total	47	30	77

^a Correlation between the CSF-restricted pattern and pleocytosis was found to be significant (two-tailed P value, 0.07114) according to Fisher's Exact Test.

^b The normal range of cells in control CSF samples is 1 to 8 cells per μ l.

- in the diagnosis of active pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **142**:380-384.
10. Clark, W. C., J. C. Metcalf, M. S. Muhlbauer, F. C. Dohan, and J. H. Robertson. 1986. *Mycobacterium tuberculosis* meningitis: a report of twelve cases and a literature review. *Neurosurgery* **18**:604-610.
 11. Closs, O., M. Harboe, N. X. Axelsen, K. Bunch-Christensen, and M. Magnusson. 1980. The antigens of *Mycobacterium bovis* strain BCG, studied by crossed immunoelectrophoresis: a reference system. *Scand. J. Immunol.* **12**:249-263.
 12. Cocito, C. 1991. Properties of the mycobacterial antigen complex A60 and its applications to diagnosis and prognosis of tuberculosis. *Chest* **100**:1687-1693.
 13. Cocito, C., M. C. Baelden, and C. Benoit. 1987. Immunological properties of antigen 60 of BCG. Induction of humoral and cellular immunity reactions. *Scand. J. Immunol.* **25**:579-585.
 14. Cocito, C., and F. Vanlinden. 1986. Preparation and properties of antigen 60 from *Mycobacterium bovis* BCG. *Clin. Exp. Immunol.* **66**:262-272.
 15. Daniel, T. M. 1987. New approaches to the rapid diagnosis of tuberculous meningitis. *J. Infect. Dis.* **155**:599-602.
 16. Daniel, T. M., and S. M. Debanne. 1987. The serodiagnosis of tuberculosis and other mycobacterial diseases by enzyme-linked immunosorbent assay. *Am. Rev. Respir. Dis.* **135**:1137-1151.
 17. Daniel, T. M., S. M. Debanne, and F. Vanderkuyp. 1985. ELISA using *M. tuberculosis* antigen 5 of PPD for the serodiagnosis of tuberculosis. *Chest* **88**:388-392.
 18. De Kesel, M., P. Gilot, M. Coene, and C. Cocito. 1992. Composition and immunological properties of the protein fraction of A36, a major antigen complex of *Mycobacterium paratuberculosis*. *Scand. J. Immunol.* **36**:201-212.
 19. Dorries, R., and V. Ter Meulen. 1984. Detection and identification of virus-specific IgG in unconcentrated cerebrospinal fluid by immunoblot technique. *J. Neuroimmunol.* **7**:77-89.
 20. Fabre, I., O. L'Homme, M. Brunetaud, G. Michel, and C. Cocito. 1986. Chemical composition of antigen 60 from *Mycobacterium bovis* BCG. *Scand. J. Immunol.* **24**:591-602.
 21. Gevaudan, M. J., C. Bollet, D. Charpin, M. N. Mallet, and P. De Micco. 1992. Serological response of tuberculosis patients to antigen 60 of BCG. *Eur. J. Epidemiol.* **8**:666-676.
 22. Grange, J. M. 1984. The humoral immune response in tuberculosis: its nature, biological role and diagnostic usefulness. *Adv. Tuberc. Res.* **21**:1-78.
 23. Gulletta, E., M. Del Pezzo, A. Sanduzzi, F. Bariffi, and I. Covelli. 1988. Serodiagnosis survey of tuberculosis by a new ELISA method. *Eur. J. Epidemiol.* **4**:331-334.
 24. Harboe, M., O. Closs, B. Bjorvatn, and G. Bjune. 1977. Antibodies against BCG antigen 60 in mycobacterial infection. *Br. Med. J.* **2**:430-433.
 25. Harboe, M., H. Wiker, R. Duncan, M. Garcia, T. Dukes, et al. 1990. Protein G-based enzyme-linked immunosorbent assay for anti MPB 70 antibodies in bovine tuberculosis. *J. Clin. Microbiol.* **28**:913-921.
 26. Hernandez, R., O. Munoz, and H. Guiscafre. 1984. Sensitive enzyme immunoassay for early diagnosis of tuberculous meningitis. *J. Clin. Microbiol.* **20**:533-535.
 27. Huygen, K., J. P. Van Vooren, M. Turneer, R. Bosmans, P. Dierckx, and J. De Bruyn. 1988. Specific lymphoproliferation, gamma interferon production, and serum immunoglobulin G directed against a purified 32 kDa mycobacterial protein antigen (P32) in patients with active tuberculosis. *Scand. J. Immunol.* **27**:187-194.
 28. Ikegaki, N., and R. H. Kennett. 1989. Glutaraldehyde fixation of the primary antibody-antigen complex on nitrocellulose paper increases the overall sensitivity of immunoblot assay. *J. Immunol. Methods* **124**:205-210.
 29. Kadival, G. V., T. B. M. S. Mazarelo, and S. D. Chaparas. 1986. Sensitivity and specificity of enzyme-linked immunosorbent assay in the detection of antigen in tuberculous meningitis cerebrospinal fluids. *J. Clin. Microbiol.* **23**:901-904.
 30. Kadival, G. V., A. M. Samuel, T. B. M. S. Mazarelo, and S. D. Chaparas. Radioimmunoassay for detecting *Mycobacterium tuberculosis* antigen in cerebrospinal fluids of patients with tuberculous meningitis. *J. Infect. Dis.* **155**:608-611.
 31. Kalish, S. B., R. C. Radin, D. Levitz, C. R. Zeiss, and J. P. Phair. 1983. The enzyme-linked immunosorbent assay method for IgG antibody to purified protein derivative in cerebrospinal fluid of patients with tuberculous meningitis. *Ann. Intern. Med.* **99**:630-633.
 32. Kaneko, K., O. Onodera, T. Miyatake, and S. Tsuji. 1990. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. *Neurology* **40**:1617-1618.
 33. Kennedy, D. H., and R. J. Fallon. 1979. Tuberculous meningitis. *JAMA* **241**:264-268.
 34. Kinnman, J., H. Link, and A. Fryden. 1981. Characterization of antibody activity in oligoclonal immunoglobulin G synthesized within the central nervous system in a patient with tuberculous meningitis. *J. Clin. Microbiol.* **13**:30-35.
 35. Krambovitis, E. 1987. Serodiagnosis of tuberculosis in perspective. *Serodiagn. Immunother.* **1**:7-19.
 36. Krambovitis, E., M. B. McIlmurray, P. E. Lock, W. Hendrickse, and H. Holzel. 1984. Rapid diagnosis of tuberculous meningitis by latex particle agglutination. *Lancet* **ii**:1229-1231.
 37. Luxton, R. W., P. Patel, G. Keir, and E. J. Thompson. 1989. A micro-method for measuring total protein in cerebrospinal fluid by using benzethonium chloride in microtiter plate wells. *Clin. Chem.* **35**:1731-1734.
 38. Nassau, E., E. R. Parsons, and G. D. Johnson. 1976. The detection of antibodies to *Mycobacterium tuberculosis* by microplate enzyme-linked immunosorbent assay (ELISA). *Tubercle* **57**:67-70.
 39. Sada, E., G. M. Ruiz-Palacios, Y. López-Vidal, and S. Ponce de Leon. 1983. Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. *Lancet* **ii**:651-652.
 40. Shankar, P., N. Manjunath, K. K. Mohan, K. Prasad, M. Behari Shrinivas, and G. K. Ahuja. 1991. Rapid diagnosis of tuberculous meningitis by polymerase chain reaction. *Lancet* **337**:5-7.
 41. Sindić, C. J. M., D. Boucquey, M. P. Van Antwerpen, M. C. Baelden, C. Laterre, and C. Cocito. 1990. Intrathecal synthesis of anti-mycobacterial antibodies in patients with tuberculous meningitis. An immunoblotting study. *J. Neurol. Neurosurg. Psychiatry* **53**:662-666.
 42. Vandvik, B., B. Sköldenberg, M. Forsgren, G. Stiernstedt, S. Jeansson, and E. Norrby. 1985. Long-term persistence of intrathecal virus-specific antibody responses after herpes simplex virus encephalitis. *J. Neurol.* **231**:307-312.
 43. Vartdal, F., B. Vandvik, and E. Norrby. 1982. Intrathecal synthesis of oligoclonal IgG, IgA and IgM virus-specific antibodies in a case of varicella-zoster meningoencephalitis. *J. Neurol. Sci.* **57**:121-132.
 44. Watt, G., G. Zaraspe, S. Bautista, and L. W. Laughlin. 1988. Rapid diagnosis of tuberculous meningitis by using an enzyme-linked immunosorbent assay to detect mycobacterial antigen and antibody in cerebrospinal fluid. *J. Infect. Dis.* **158**:681-686.
 45. Wright, G. L., Jr., L. F. Affronti, and M. Reich. 1972. Characterization and comparison of mycobacterial antigens by two-dimensional polyacrylamide gel electrophoresis. *Infect. Immun.* **5**:482-490.
 46. Zeiss, C. R., S. B. Kalish, and K. S. Ehrlich. 1984. IgG antibody to purified protein derivative by ELISA in the diagnosis of pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **130**:845-848.