Clin. exp. Immunol. (1991) **84**, 515–521 ADONIS 000991049100180X

Antigens of *Mycobacterium leprae* in the cerebrospinal fluid of leprosy patients: detection by monoclonal-antibody-based sandwich immunoradiometric assay and avidin/biotin immunoblotting

S. A. PATIL, P. TYAGI, K. KATOCH*, SREEVATSA† & U. SENGUPTA
*Departments of Immunology, *Medicine and †Experimental Leprosy Central JALMA Institute for Leprosy, Agra, Uttar Pradesh, India*

(Accepted for publication 2 January 1991)

SUMMARY

Mycobacterium leprae antigens could be detected in the cerebrospinal fluid (CSF) of patients with leprosy, using a monoclonal-antibody-based sandwich immunoradiometric assay (SIRMA). Antigens of 12 kD, 35 kD and 30–40 kD were detected using ML06, ML04, and ML34 monoclonal antibodies, respectively. The 30–40-kD polysaccharide antigen, although present in larger amounts in *M. leprae* than the 12-kD and 35-kD protein antigens, was found in the CSF of comparatively fewer subjects. The antigen capture assay has been found sensitive to the level of nanograms. Avidin–biotin-based immunoblotting using pooled leprosy sera detected a larger number of antigens than using anti-*M. leprae* antisera raised in rabbits. The immunoblotting of CSF samples revealed about three antigens in the region of 100–160 kD and three more in the region of 45–60 kD as probed by leprosy sera. This study has for the first time revealed the presence of *M. leprae* antigens in the CSF of leprosy patients and the probable involvement of the central nervous system in leprosy.

Keywords *Mycobacterium leprae* antigens cerebrospinal fluid leprosy SIRMA

INTRODUCTION

Leprosy is a chronic infectious disease caused by *M. leprae*. The pathogen affects primarily nerves and skin, and at times causes deformity. Unlike any other mycobacteria, *M. leprae* has been shown to have selective affinity to nerve cells (Mukherjee & Antia, 1985). *M. leprae* could also be detected in axons of peripheral nerves in patients with lepromatous leprosy and in mice with leprosy (Boddingius, 1974). Presence of *M. leprae* antigen has also been detected in nerve biopsy of leprosy patients (Mshana *et al.*, 1983). Although leprosy is primarily a disease of nerves, a very high amount of antigenic load is noticed in the blood of patients with severe forms of leprosy (Drutz, Chen & Lu, 1972), yet nothing is known about the involvement of central nervous system in this disease. Under normal conditions the blood–brain barrier (BBB) plays a vital role, which allows the flow of only essential nutrients and excludes IgG or IgM antibodies (Griffin & Giffels, 1982). However, the brain barrier mechanisms are of physicochemical nature and are liable to be damaged or altered in certain pathological conditions (Vaidya, Badacha & Raizada, 1983). In most of the chronic infections BBB has been reported to be damaged (Quagliarello, Long & Scheld, 1986). Among the mycobacterial infections, *M. tuberculosis* antigen has been detected in the cerebrospinal fluid (CSF) of patients with tuberculous meningitis (TBM) (Chandramukhi *et al.*, 1985; Kadival *et al.*, 1987; Daniel & Debanne, 1987) indicating the involvement of central nervous system (CNS) in the diseased individuals.

In inflammatory diseases, antigen from the circulation is reported to enter the CSF (Kadurugamua, Hengstler & Zak, 1989). In experimental situation, it has been demonstrated that serum proteins have an influx into the CSF (Frydan, Link & Norrby, 1978; Laskin & Griffin, 1987; Quagliarello *et al.*, 1986). In leprosy, inflammation is quite common in reactions, and millions of bacilli are found in the blood and tissues (Drutz *et al.*, 1972). It is probable that the bacilli or their products may cross the BBB of the host. If such a mechanism is present in leprosy, it may lead to the pathological changes in this primary disease of nerves. In experimental situations, *M. leprae* has been shown to cross the BBB in thymectomized, irradiated mice (Vaidya *et al.*, 1970) and *M. lepraemurium* in the infection in ordinary mice (Vaidya & Rees, 1975). In both situations the presence of the bacilli could be detected in sections of brain. Such a study is not plausible in human subjects. However, by taking the CSF sample from leprosy patients the probable presence of *M. leprae* or *M. leprae* products can be studied.

The present study was undertaken to detect the presence of *M. leprae* antigens in the CSF of leprosy patients, by using

Correspondence: Shripad A. Patil, Ph.D., Department of Immunology, Central JALMA Institute for Leprosy, Taj Ganj, P.O. Box-31, Agra 282001 (U.P.), India.

monoclonal antibody (MoAb) based sensitive immunoassays and immunoblotting.

SUBJECTS AND METHODS

Subjects

Thirty-four patients with active lepromatous leprosy admitted to the Central JALMA Institute for Leprosy, Agra, India, were selected for the study. Clinical classification of the disease was done according to Ridley & Jopling (1966). CSF was obtained aseptically from these subjects by lumbar puncture and stored at −70°C. Fourteen samples of CSF from subjects with tuberculosis meningitis (TBM) were procured from the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. CSF from 10 subjects admitted at S.N. Medical College, Agra, for complications other than microbial infection (accident and operation cases), served as controls in the study.

Monoclonal antibodies (MoAbs)

The MoAbs ML06, ML04 and ML34 which bind to epitopes on 12 kD, 35 kD (proteins) and 30–40 kD (polysaccharide) soluble components of *M. leprae*, respectively (Ivanyi *et al.*, 1983), were labelled with ^{125}INa (Barc, Bombay, India), by the iodogen method (Fraker & Speck, 1978).

M. leprae *antigen*

Armadillo-derived, purified cell-free extract of *M. leprae* soluble antigen (Draper & Rees, 1975) was procured from the IMMLEP (WHO) bank, London, UK.

Sandwich immunoradiometric assay (SIRMA)

This assay was done as described elsewhere (Patil *et al.*, 1990). In this solid-phase antigen capture assay, antibody molecules are coated on a solid phase and the antigen from the test sample is captured by the adhered antibody molecule. Another antibody molecule which is radiolabelled is used for probing the captured antigen. The principle in this assay is that the antigen should have at least two sites to combine with two antibody molecules.

Flexible microtitre plates (U.96, Dynatech) were coated with 50 μl/well of any of the MoAbs by overnight incubation at 4°C in a moist chamber. Unbound antibody was removed, wells were dried by patting the plates on tissue paper. Free binding sites of the wells were blocked with 100 μl of 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 2 h at room temperature. Excess BSA was removed and wells were dried. Test samples (50 μl of CSF) were applied in triplicates and plates were incubated for 2 h at 37°C in a moist chamber. Plates were then washed with PBS and 50 μl of corresponding ^{125}I-labelled MoAb were added to each well. The plates were incubated again for two hours, washed three times with PBS, dried and radioactive counts of each well were counted in a gamma counter (N.E. 1600, N.E., Edinburgh, UK).

CSF samples giving counts above the mean + 2 s.d. of CSF from controls were taken as positive for the antigen.

Antigen standard curve

Standard samples with known concentrations of antigens were prepared by incorporation of *M. leprae* antigen in the pooled CSF sample of control subjects. These samples were subjected to SIRMA using each MoAb and thus standard curves were constructed.

*Anti-*M. leprae *antibodies*

Anti-*M. leprae* antibodies in CSF against an antigenic component on 35-kD molecule was detected using MoAb ML04 by immunoradiometric antibody competition assay by the method of Hewitt *et al.* (1982).

SDS–PAGE and immunoblotting

SDS–PAGE was carried out according to Laemmli (1970) with some modifications: 12% homogeneous gels were used for electrophoresis in a continuous buffer system. The CSF samples were prepared by mixing with equal volumes of sample buffer consisting of 5 ml of 10% SDS, 2·5 ml of 0·5 M Tris (pH 6·8), 5 ml distilled water, 2·5 ml of glycerol, 5 mg of di-thiothreitol and 0·2 ml of 5% bromophenol blue. The samples were heated in a water-bath at 100°C for 2 min and were used for electrophoresis after cooling. Twenty microlitres each of these CSF samples (CSF and sample buffer) and molecular weight markers (Pharmacia, Uppsala, Sweden) were subjected to electrophoresis at a constant current using Pharmacia PAGE apparatus. Visualization of proteins was done by staining the gel with PAGE blue (BDH, Poole, UK) followed by destaining in a mixture of 25% methanol and 7% acetic acid, and the gel was restained by silver staining (Wray *et al.*, 1981).

Immunoblotting was done according to Towbin, Staehlin & Gordon (1979) using Pharmacia electroblotting apparatus. CSF samples from leprosy and TBM subjects were blotted onto nitrocellulose paper and were allowed to react with lepromatous leprosy sera or with anti-*M. leprae* antibodies raised in rabbits, and enzyme reaction was performed by the avidin–biotin technique (Guesdon, Ternyck & Avrameas, 1979) using 4-chloronapthol substrate.

RESULTS

The *M. leprae* antigens of 12 kD, 35 kD and 30–40 kD were captured by using ML06, ML04 and ML34 MoAbs, respectively. Standard curves constructed for 35-kD and 30–40-kD antigens could work in a dose-dependent manner (Fig. 1a, b). However, a standard curve could not be constructed for the 12-kD antigen. This could be because of the presence of a single epitope recognizing ML06 antibody on the 12-kD molecule (Patil *et al.*, 1990).

The 12-kD antigen

Twenty-four of the 34 (71%) subjects with leprosy and six of 14 (43%) subjects with TBM were found positive for the 12-kD antigen (Table 1). None of the 10 CSF samples from controls was found positive for the antigen (Fig. 2a). The cut-off point, calculated by the meean + 2 s.d. counts shown by CSF samples obtained from controls was about 170 ct/min. The sensitivity of the assay could not be calculated because of the lack of dose-dependent behaviour of this antigen. However, the highest ct/min, 410, was recorded in a CSF sample of a subject with TBM.

The 35-kD antigen

Thirty of the 34 (90%) subjects with leprosy and 10 of the 14 (71%) subjects with TBM were found positive for the 35-kD antigen (Table 1). None of the 10 CSF samples from the controls was found positive by the assay (Fig. 2b). The cut-off point calculated for this antigen was about 176 ct/min. The assay was sensitive to the level of 48 ng. The highest level of antigen

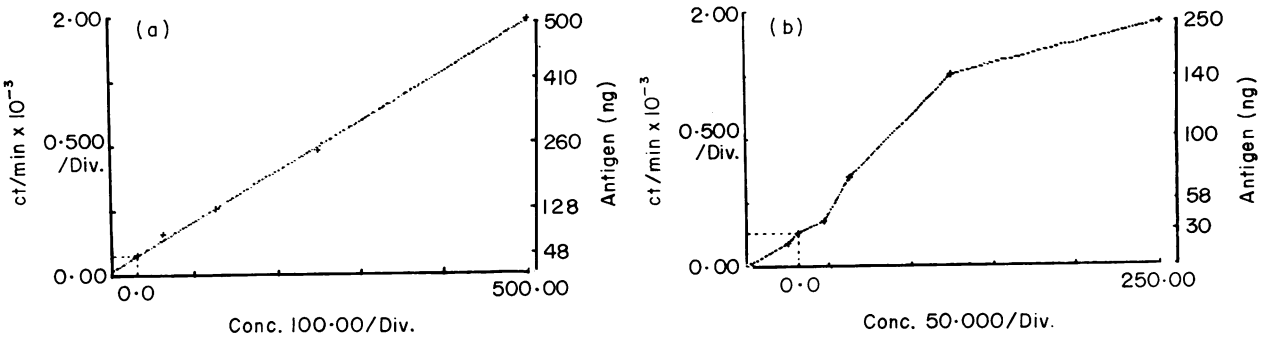

Fig. 1. Standard curve constructed by incorporating soluble *M. leprae* antigen in pooled control CSF. (a) Standard curve for 35-kD antigen of *M. leprae*. The sensitivity level of the assay calculated on the basis of CPM is 44 ng; (b) standard curve for 30–40-kD polysaccharide *M. leprae* antigen. The sensitivity level of the assay is 28 ng as calculated on the basis of ct/min.

Table 1. Analysis of antigen positivity for 12-kD, 35-kD and 30–40-kD components of *M. leprae* in the CSF of subjects with leprosy, tuberculous meningitis (TBM) and controls as seen by monoclonal-antibody-Sandwich immunoradiometric assay

	Antigen positivity (%)			Number positive for (%)		
Subjects	12 kD	35 kD	30–40 kD	All the three antigens	Any of the antigens	None of the antigens
Leprosy ($n=34$)	24 (71)	30 (88)	14 (41)	13 (38)	31 (91)	3 (9)
TBM ($n=14$)	6 (43)	10 (71)	2 (14)	2 (14)	10 (71)	4 (29)
Controls ($n=10$)	0 (0)	0 (0)	1 (10)	0 (0)	1 (10)	9 (90)

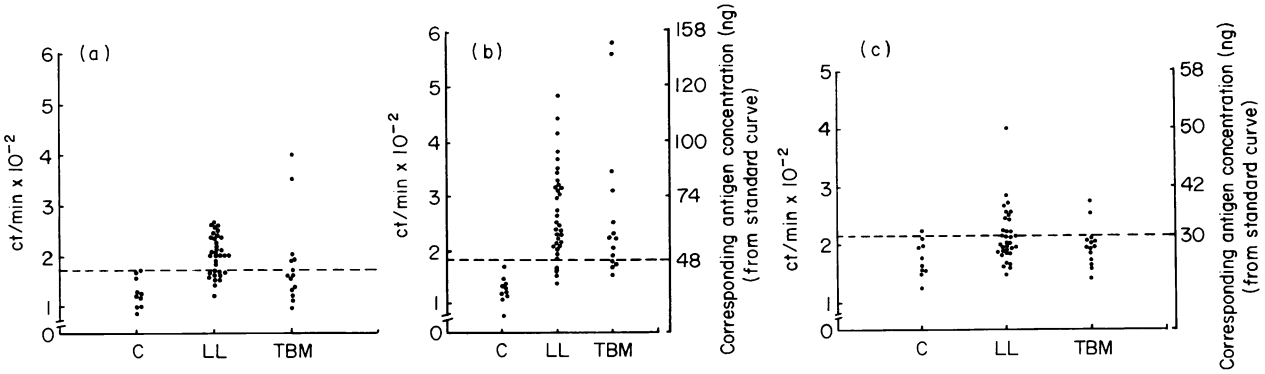

Fig. 2. Presence of *M. leprae* antigens in the CSF, detected by SIRMA: (a) 12-kD *M. leprae* protein antigen in CSF samples. Antigen concentration against the ct/min could not be calculated for this antigen molecule with single epitope; (b) 35-kD *M. leprae* protein antigen in CSF samples. – – –, Cut-off point; (c) 30–40-kD *M. leprae* polysaccharide antigen in CSF samples. – – –, Cut-off point. C, controls ($n=10$); LL, lepromatous leprosy ($n=34$); TBM, tuberculous meningitis ($n=14$).

(3·2 μg/ml) could be recorded in the CSF sample of a subject with TBM.

The 30–40-kD antigen

CSF samples from 14 of the 34 (41%) subjects with leprosy and two of the 14 (14%) subjects with TBM were found positive for the 30–40-kD polysaccharide antigen (Table 1). One of the CSF samples from the control group also showed antigen positivity by the assay (Fig. 2c). The sensitivity of the assay was to the level of 30 ng. The highest level of antigen (1·2 μg/ml) was recorded in a CSF sample of a subject with leprosy.

The three tested antigens together could be detected in about 38% of leprosy patients; any one of the three antigens could be detected in 91% of the subjects and about 9% of the subjects did not show antigen positivity for any of the above three tested antigens (Table 1). A significant correlation was observed $P<0·001$, $r=0·934$ in the 12-kD protein antigen positivity detected by *M. leprae*-specific MoAb ML06 and 35-kD protein antigen positivity detected by quasi-specific MoAb ML04 (Fig. 3a). However, the correlation was insignificant when the 12-kD and 35-kD antigen positivities were compared with the positivity of 30–40-kD mycobacterial cross-reactive polysac-

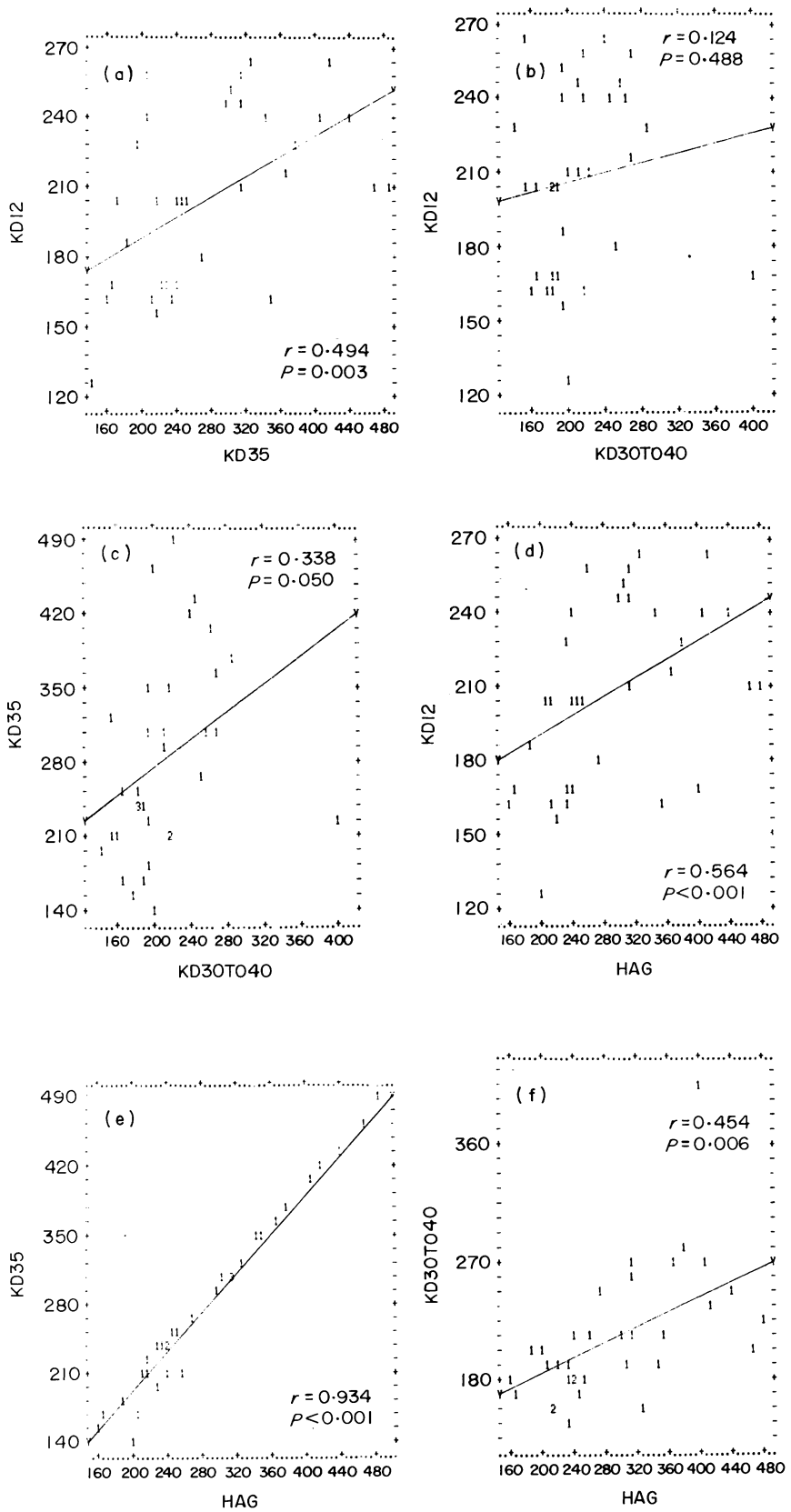

Fig. 3. Comparison among the 12-kD, 35-kD and 30–40-kD components of *M. leprae* in CSF and with the presence of highest level of antigen (HAG). (a) Correlation of the 12-kD and 35-kD antigens; (b) 12-kD and 30–40-kD antigens; (c) 35-kD and 30–40-kD antigens; (d) correlation of 12-kD antigen with HAG; (e) correlation of 35-kD antigen with HAG; (f) correlation of 30–40-kD antigen with HAG.

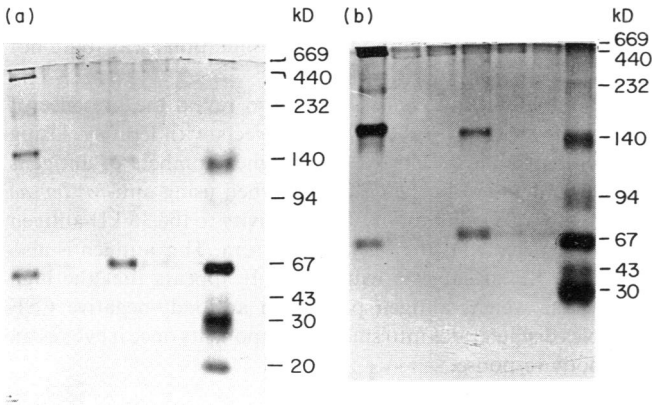

Fig. 4. SDS–PAGE pattern of CSF samples. (a) PAGE blue staining of gel; (b) silver staining. Lanes 1 and 7, high and low molecular weight markers, respectively; lanes 2, 3 and 4, CSF samples from leprosy subjects; lanes 5 and 6, CSF samples from TBM subjects.

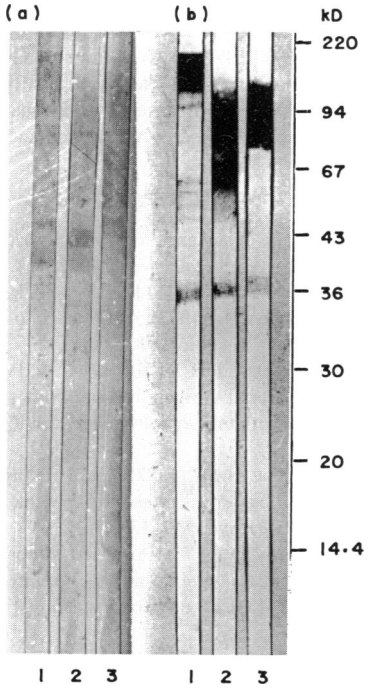

Fig. 5. Immunoblotting of CSF samples: Probing of *M. leprae* antigens using (a) anti-*M. leprae* antibodies raised in rabbit; and (b) using high titered pooled leprosy sera. Lanes 1, antibody-negative pooled CSF sample from leprosy patients; lanes 2, antibody positive pooled CSF sample from leprosy patients; lanes 3, pooled CSF sample from TBM subjects.

charide antigen ($P > 0{\cdot}05$, $r = 0{\cdot}124$, and $P = 0{\cdot}05$, $r = 0{\cdot}338$, respectively) (Fig. 3b, c).

The ct/min observed in detection of 12-kD, 35-kD and 30–40-kD antigens when correlated with the highest level of antigen (highest ct/min of the three tested antigens) showed significant correlation with 12-kD antigen ($P < 0{\cdot}001$, $r = 0{\cdot}564$) and highly significant correlation with the 35-kD antigen ($P < 0{\cdot}001$, $r = 0{\cdot}934$) (Fig. 3d, e). However, the ct/min observed for the 30–40-kD antigen did not correlate well ($P > 0{\cdot}005$, $r = 0{\cdot}454$) with the highest level of antigen (Fig. 3f).

SDS–PAGE and immunoblotting

PAGE patterns of CSF samples from subjects with leprosy and TBM revealed three to six bands by PAGE blue (Fig. 4a) and by silver staining (Fig. 4b). The antigenic nature of these bands was revealed by avidin–biotin immunoblotting.

Using rabbit anti-*M. leprae* antibody, an antigenic band in the region of 120–160 kD, one band in the region of 90 kD and two bands in the region of 40–48 kD could be detected in an anti-35-kD antibody-negative CSF sample of a leprosy subject (Fig. 5a). In an anti-35-kD antibody-positive leprosy CSF sample, two antigenic bands in the region of 40–44 kD were noticed. However, in the CSF sample of a TBM subject one faint antigenic band in the region of 40–48 kD and one faint broad antigenic band in the region of 50–70 kD could be detected.

Using high-titre pooled leprosy sera, antigens of 120–160 kD, 100 kD and four weak antigens in the range of 45–60 kD could be seen in an anti-35-kD antibody-negative pooled CSF sample (Fig. 5b). The anti-35-kD antibody-positive CSF sample from subjects with leprosy showed a thick antigenic band in the region of 78–120 kD and another thick antigenic band in the region of 60–75 kD. The antigenic band of 78–120 kD was also shared by the CSF sample from a TBM subject who was negative for anti-35-kD antibody. A weak antigen in the region of 36 kD was shared by both anti-35-kD antibody-positive and antibody-negative CSF samples of leprosy patients and TBM patients.

DISCUSSION

In the present study CSF samples have been screened for the *M. leprae* antigens to find out whether these antigens get sequestered in the CNS. In experimental situations, the BBB is reported to be impaired in animals with *M. leprae* infection (Vaidya *et al.*, 1970). Such a condition has also been reported to occur in other diseases like chronic trypanosomal infection (Waitumbi, Sayer & Gould, 1986), acute and chronic AIDS (Shaw, Harper & Hahn, 1985; Goudsmit, Paul & Lange, 1986), and in TBM (Chandramukhi *et al.*, 1985; Kadival *et al.*, 1987; Daniel & Debanne, 1987). In diseases such as AIDS, HIV is selectively sequestered in the brain tissue through a special transport system (Shaw *et al.*, 1985). As the presence of acid–fast bacilli could not be determined in the CSF of leprosy patients, it is not clear whether such a mechanism exists in leprosy. An earlier study has also failed to detect acid-fast bacilli in the CSF of leprosy patients (Vaidya *et al.*, 1970). However, here we detected for the first time *M. leprae* antigen in the CSF of leprosy patients. Small peptides normally do not cross the BBB, unless there is a specific transport system for them (Pardridge, 1983). In diseases such as Down's syndrome (Glenner, Henry & Fujihara, 1981) and Alzheimer's disease (Shirahama, Skinner & Westermark, 1982) abnormal proteins seem to be transported selectively through the BBB. In leprosy further studies are warranted to understand whether the *M. leprae* antigens in CSF of leprosy patients are transported selectively through the BBB or whether this occurs due to an impairment of the BBB. However, the role of the acquired presence of *M. leprae* antigen in CSF of leprosy patients is not clear.

Our MoAb-based sandwich immunoradiometric antigen capture assay is highly sensitive and could detect the *M. leprae* antigens to the level of nanograms. This assay works in a dose-dependent manner for 35-kD protein antigen and 30–40-kD polysaccharide antigen in the construction of standard curve. Polysaccharide antigens by nature are expected to contain repetitive antigenic epitopes which enable the sandwich assay to work. The 35-kD protein antigen also seems to contain at least two antigenic epitopes and hence behaves in a dose-dependent manner in the standard curve. In an earlier study this protein antigen was shown to have an unusual precipitable property by MoAb ML04 as seen by crossed immunoelectrophoresis (Harboe & Ivanyi 1987). However, a standard curve could not be constructed for the 12-kD antigen by the sandwich antigen capture assay. This may be because of the presence of single antigenic epitope-reacting MoAb ML06 (Patil *et al.*, 1990). However, the 12-kD antigen from the CSF of leprosy patients could be detected by the sandwich antigen capture assay, suggesting the differences between *in vivo*-processed antigen and armadillo-derived *M. leprae* sonicated antigen with regard to the distribution of antigenic epitopes.

The 12-kD component of *M. leprae*, which is a weak immunogen (Patil *et al.*, 1990), could be detected in about 70% of CSF samples from subjects with leprosy, and the 35-kD antigenic component, which is a strong immunogen (Ivanyi *et al.*, 1983), could be detected in about 90% of the samples. This antigen (35 kD) could be detected in almost all the CSF samples showing positivity for any of the three tested antigens. The 30–40-kD polysaccharide antigen, which is a weak immunogen especially in tuberculoid leprosy (Mwatha *et al.*, 1988; Patil *et al.*, 1990), could be detected in only about 40% of the leprosy subjects. This polysaccharide antigen is notably in higher quantities in *M. leprae* than the two other studied antigens (Ivanyi, Morris & Keen, 1985).

Statistical analysis of the presence of *M. leprae* antigens of varied molecular weight was performed to see whether the size of the antigen molecules affected the passing of the antigens through the BBB. However, it was found that it was mainly the nature of antigen, as opposed to its size that was responsible for the phenomenon. Antigens of protein nature seem to traverse more often through the BBB than the polysaccharide antigen. A positive correlation was also observed for the presence of mixed protein antigens in the CSF. However, with polysaccharide antigen the correlation with other antigens was insignificant. Therefore, it seems that protein antigens pass through the BBB preferentially.

Leprosy and tuberculosis are chronic diseases caused by related mycobacterial species that share almost common endemic regions in the world (Bothamley *et al.*, 1987). In India, in various leprosy endemic regions, the endemic population of tuberculosis are likely to be exposed to leprosy infection and vice versa (Fine, 1984). The present finding of *M. leprae*-specific antigen in CSF of TBM patients is probably due to concomittant infection with *M. leprae*. In a similar study, the presence of antibodies to specific component of *M. tuberculosis* has also been shown in the serum of leprosy patients (Bothamley *et al.*, 1987). However, in the present study none of the TBM patients had symptoms of leprosy.

Two of the CSF samples from TBM subjects were found positive for all the three tested antigens, including the *M. leprae*-specific one. They also possessed specific antibodies such as antibodies against the immunodominant 35-kD component, as detected by a MoAb-antibody-based inhibition assay (data not shown).

Immunoblotting studies could also reveal the presence of *M. leprae* antigens in the CSF of subjects with leprosy. Using pooled lepromatous leprosy sera, higher numbers of antigens could be detected in the CSF than when using anti-*M. leprae* antibodies raised in rabbits. The reactivity to the 36-kD antigen could be detected using only leprosy sera. This antigen is also seen in CSF of subjects with TBM. It appears that the high molecular weight antigen present in antibody-negative CSF samples disintegrates into smaller components once it evokes an antibody response.

ACKNOWLEDGMENTS

We thank Dr R. J. W. Rees, IMMLEP (WHO) bank, London, and Dr J. Ivanyi, MRC Unit, Hammersmith Hospital, London, for providing us with soluble *M. leprae* antigen and globulin fractions of monoclonal antibodies, respectively. Drs A. Chandramukhi, NIMHANS, Bangalore, and B. B. Maheshwari, S.N. Medical College, Agra, are thanked for providing the specimens from tuberculous meningitis and healthy control subjects, respectively, for the study. We are also grateful to Dr H. Srinivasan for critical review of the manuscript, and Drs M. J. Colston, R. Barnetsan and K. P. W. J. McAdam for helpful discussions. The kind help in statistical analysis by Mr A. S. Bhatia, the secretarial assistance by Mr Anil Kumar Chopra, technical assistance by Mr C. R. Pal and photography by Mr Hari Om Agarwal are acknowledged.

REFERENCES

Boddingius, J. (1974) The occurrence fo *Mycobacterium leprae* within axons of peripheral nerves. *Acta neuropath.* **27,** 257.

Bothamley, G., Beck, J.S., Agusni, I., Ilias, M.I., Kardjito, T., Grange, J.M. & Ivanyi, J. (1987) Antibodies to *Mycobacterium tuberculosis* in leprosy. *Lancet*, **i,** 1098.

Chandramukhi, A., Allen, P.R.J., Keen, M. & Ivanyi, J. (1985) Detection of mycobacterial antigen and antibodies in the cerebrospinal fluid of patients with tuberculous meningitis. *J. med. Microbiol.* **20,** 239.

Daniel, T.M. & Debanne, S.M. (1987) The serodiagnosis of tuberculosis and other mycobacterial diseases by enzyme-linked immunosorbent assay. *Am. Rev. respir. Dis.* **135,** 1137.

Draper, P. & Rees, R.J.W. (1975) Proposed system for preparing purified suspensions of *M. leprae* from tissues of infected armadillos. Report of the second IMMLEP task force meeting, protocol no.2/75. *Leprosy Rev.* **47,** 320.

Drutz, D.J., Chen, T.S.N. & Lu, W.H. (1972) The continuous bacteremia of lepromatous leprosy. *N. Engl. J. Med.* **287,** 159.

Fine, P. (1984) Leprosy and tuberculosis: an epidemiological comparison. *Tubercle*, **65,** 137.

Fraker, P.J. & Speck, J.C. (1978) Protein and cell membrane iodination with sparingly soluble chloramide, 1,3,4,6-tetrachloro-3α6αdiphenyl glycoluril. *Biochem. Biophys. Res. Comm.* **80,** 849.

Fryden, A., Link, H. & Norrby, E. (1978) Cerebrospinal fluid and serum immunoglobulins and antibody titers in mumps meningitis and aseptic meningitis of other etiology. *Infect. Immun.* **21,** 852.

Glenner, G.G., Henry, J.H. & Fujihara, S. (1981) Congophilic angiopathy in the pathogenesis of Alzheimer's degeneration. *Ann. Pathol.* **1,** 120.

Goudsmit, J., Paul, D.A. & Lange, J.M.A. (1986) Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet*, **ii,** 177.

Griffin, D.E. & Giffels, J. (1982) Study of protein characteristics that influence entry in to cerebrospinal fluid of normal mice and mice with encephalitis. *J. clin. Invest.* **70,** 289.

GUESDON, J., TERNYCK, T. & AVRAMEAS, S. (1979) The use of avidin biotin interaction in immunoenzymatic techniques. *J. Histochem. Cytochem.* **27,** 1131.

HARBOE, M. & IVANYI, J. (1987) Analysis of monoclonal antibodies to *Mycobacterium leprae* by crossed-immunoelectrophoresis. *Scand. J. Immunol.* **25,** 133.

HEWITT, J., COATES, A.R.M., MITCHISON, D.A. & IVANYI, J. (1982) The use of murine monoclonal antibodies without purification of antigen in the serodiagnosis of tuberculosis. *J. immunol. Methods*, **55,** 205.

IVANYI, J., MORRIS, J.A. & KEEN, M. (1985) Studies with monoclonal antibodies to mycobacteria. In *Monoclonal Antibodies Against Bacteria* (ed. by A.J.L. Macario & E.C. Macario) p. 59. Academic Press, New York.

IVANYI, J., SINHA, S., ASTON, R., CUSSEL, D., KEEN, M. & SENGUPTA, U. (1983) Definition of species specific and cross-reactive antigen determinants of *Mycobacterium leprae* using monoclonal antibodies. *Clin. Exp. Immunol.* **52,** 528.

KADIVAL, G.V., SAMUEL, A.M., MAZARELO, T.B.M.S. & CHAPRAS, S.D. (1987) Radio immunoassay for detecting *Mycobacterium tuberculosis* antigen in CSF of patients with tuberculous meningitis. *J. infect. Dis.* **155,** 608.

KADURUGAMUA, J.L., HENGSTLER, B. & ZAK, O. (1989) Cerebrospinal fluid profile in experimental pneumococcal meningitis and its alteration by amplicillin and anti-inflammatory agents. *J. infect. Dis.* **159,** 26.

LAEMMLI, U.K. (1970) Clevage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, **227,** 680.

LASKIN, O.L. & GRIFFIN, D.E. (1987) Changes in cerebrospinal fluid cells, IgG and IGA during herpes simplex virus encephalitis in rabbits. *J. Neuroimmunol.* **14,** 283.

MSHANA, R.N., HUMBER, D.P., HARBOE, M. & BELEHU, A. (1983) Demonstration of mycobacterial antigens in nerve biopsies from leprosy patients using peroxidase anti-peroxidase immunoenzyme technique. *Clin. Immunol. Immunopathol.* **29,** 359.

MUKHERJEE, R. & ANTIA, N.H. (1985) Adherence of *M. leprae* to schwann cells *in vitro*. A specific phenomenon. *IRCS Med. Sci.* **13,** 853.

MWATHA, J., MORENO, C., SENGUPTA, U., SINHA, S. & IVANYI, J. (1988) A comparative evaluation of serological assay for lepromatous leprosy. *Leprosy Rev.* **59,** 195.

PARDRIDGE, W.M. (1983) Neuropeptides and the blood brain barrier. *Annu. Rev. Phsyol.* **45,** 73.

PATIL, S.A., GIRHDAR, B.K., SINGH, K.P. & SENGUPTA, U. (1990) Detection of *Mycobacterium leprae* antigens in the sera of leprosy patients by sandwich immunoradiometric assay (SIRMA) using monoclonal antibodies. *J. clin. Microbiol.* **28,** 2792.

QUAGLIARELLO, V.J., LONG, W.J. & SCHELD, W.M. (1986) Morphologic alterations of the blood brain barrier with experimental meningitis in the rat. Temporal sequence and role of encapsulation. *J. clin. Invest.* **77,** 1084.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five group system. *Int. J. Leprosy*, **34,** 255.

SHAW, G.M., HARPER, M.E. & HAHN, B.H. (1985) HTLV III infection in brains of children and adults with AIDS encephalopathy. *Science*, **227,** 177.

SHIRAHAMA, T., SKINNER, M. & WESTERMARK, P. (1982) Senile cerebral amyloid prealbumin as a common constituent in the neuritic plaque in the neurofibrillary tangle and in the microangiopathic lesion. *Am. J. Pathol.* **107,** 41.

TOWBIN, H., STAEHLIN, T. & GORDON, G. (1979) Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets, procedure and some applications. *Proc. natl Acad. Sci. USA*, **76,** 4350.

VAIDYA, M.C. & REES, R.J.W. (1975) Observations on the brain in murine leprosy. In *Proceedings of the 10th International Congress on Anatomy, Tokyo*, p. 238.

VAIDYA, M.C., BADACHA, P. & RAIZADA, N. (1983) Central nervous system and *M. leprae* infection. In *Progress in Immunology of Leprosy* (ed. by G. P. Talwar, J. L. Turk & R. J. W. Rees), p. 304. Mayfair Press, New Delhi.

VAIDYA, M.C., PALMER, E., WEDDELL, G. & REES, R.J.W. (1970) A note on the presence of *Mycobacterium leprae* in the central nervous system of a mouse with lepromatous leprosy *J. med. Microbiol.* **3,** 194.

WAITUMBI, J.N., SAYER, P.D. & GOULD, S.S. (1986) Evidence of blood barrier permeability impairment in chronic *Trpanosoma rhodesience* infection in vervet monkey. *Trans. R. Soc. trop. Med. Hyg.* **80,** 848.

WRAY, W., BOULIKAS, T., WRAY, V.P. & HANCOCK, R. (1981) Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* **118,** 197.