Further studies of the fluorescent treponemal antibody-cerebrospinal fluid (FTA-CSF) test with a monospecific anti-IgM conjugate

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Recent studies have shown the fluorescent treponemal antibody-cerebrospinal fluid (FTA-CSF) test to be the most sensitive of the serological tests investigated for the detection of treponemal antibody in cerebrospinal fluid (CSF) (Duncan and Kuhn, 1972; Duncan, Jenkins, and Parham, 1972). The CSF specimens were obtained from humans with various stages of syphilis and from four chimpanzees before and at intervals after experimental infection with *T. pallidum*.

However, the question of *a priori* significance of the reactive FTA results in CSF still remains. When an immunologically broad spectrum conjugate is used, there is a probability of detecting treponemal IgG antibody, the origin of which may involve:

- (1) An equilibrium of antibody between the serum and CSF;
- (2) A damaged blood-brain barrier;
- (3) Local (CNS) production of antibody;
- (4) A combination of these.

Immunological studies of serum specimens by others have suggested that, since the IgM class of treponemal antibody supposedly appears earlier than that of the IgG class, the use of monospecific fluorescein labelled anti-IgM conjugate could result in earlier detection of the disease. Such a conjugate has been shown to be of value in differentiating passively transferred antibody from active syphilitic infection of the newborn (U.S. Department of Health, Education and Welfare, 1972).

The IgM class of immunoglobulin is not considered to be a constituent of normal CSF (Schultze and Heremans 1966). Under pathological conditions, however, IgM has been found in CSF associated with conditions of increased capillary permeability or local immunoglobulin production, including neurosyphilis (Laterre, Heremans, and Demanet, 1962; Oxelius, Rorsman, and Laurell, 1969).

A method combining the sensitivity of the indirect

fluorescent antibody (IFA) procedure and the specificity inherent in the use of a monospecific anti-IgM conjugate to detect a treponemal IgM antibody from within the CNS could be valuable in the early detection of neurosyphilis.

The purpose of the present study was to survey the occurrence of treponemal IgM antibody detectable by an IFA procedure in CSF specimens from humans infected with syphilis and from chimpanzees used as animal models for the study of the human disease.

Material and methods

(1) Cerebrospinal fluid

370 CSF specimens were examined. A single CSF specimen was obtained from each of 29 humans in whom a diagnosis of neurosyphilis was made by the submitting physicians. Also, according to these physicians, nine of the individuals were thought to have been inadequately treated, twelve were thought to have been adequately treated, seven had received no antisyphilitic treatment, and on one no information was available.

The remaining 341 CSF samples were collected from 39 chimpanzees: 264 specimens were obtained sequentially from 24 chimpanzees before and up to 6 years after experimental infection with *T. pallidum*, 47 specimens were obtained sequentially from five chimpanzees before and up to 3 years after experimental infection with *T. carateum*, and thirty specimens were obtained from ten chimpanzees which were not experimentally infected although five of the animals did have naturally occurring treponemal serum antibody (Kuhn, Brown, and Falcone, 1968). Nine animals were treated after infection, and thirty did not receive antitreponemal treatment.

(2) Test indicators

The fluorescein labelled conjugates for the IFA tests were obtained from commercial sources. Specificities of the conjugates were determined by IFA, gel diffusion, and immunoelectrophoretic methods by personnel of the Bacteriology Branch, Center for Disease Control (CDC), Atlanta, Georgia. The anti-IgG conjugates were predominantly anti-IgG, but did have light chain activity which reacted with IgM antibody (Hunter, 1971). At

least two lots of the anti-IgG conjugates were used in this study. The anti-IgM conjugate was found to be monospecific, and only one lot was used.

(3) Antigen

For the IFA tests a single lot of the improved *Treponema pallidum* antigen was used throughout the study (Hunter, Creighton, and Lewis, 1970).

(4) Serological tests

The FTA-CSF test was done as previously described (Duncan, Jenkins, and Parham, 1972). The FTA-CSF procedure with the anti-IgM conjugate was carried out in a manner similar to the FTA-CSF test except that the monospecific anti-IgM conjugate was substituted for the anti-IgG conjugate. An antitreponemal IgM control serum was used to titrate the anti-IgM conjugate and to establish the 1+ reading standard.

Results

A summary of the results of the FTA-CSF tests performed on the human CSF specimens is given in Table I. No treponemal IgM antibody was detected in the human specimens, but the IgG procedure

TABLE I *Test results on human CSF specimens*

Human neurosyphilis	Number of specimens	FTA-CSF IgM ^a test		FTA-CSF IgG ^b test	
		R	NR	R	NR
Untreated	7	0	7	6	1
Inadequately treated	9	0	9	7	2
Adequately treated	12	0	12	9	3
Unknown treatment	1	0	1	1	0
Total	29	0	29	23	6

^aFluorescent treponemal antibody—cerebrospinal fluid test with anti-IgM conjugate.

^bFluorescent treponemal antibody—cerebrospinal fluid test with anti-IgG conjugate.

R = Reactive.

NR = Nonreactive.

TABLE II *Test results on chimpanzee CSF specimens*

Category	FTA-ABS ^a reactivity of serum	No. of chimpanzees	FTA-CSF IgM ^b		FTA-CSF IgG ^c		
			R	NR	R	NR	NT
Infected and not treated	R	5	1	74	71	4	0
	NR	15	0	213	162	49	2
Infected and treated	R	2	0	6	5	0	1
	NR	7	0	17	13	4	0
Not infected and not treated	R	5	2	19	13	8	0
	NR	5	0	9	0	9	0
Total		39	3	338	264	74	3

^aFluorescent treponemal antibody—absorption test result on serum before experimental infection.

^bFluorescent treponemal antibody—cerebrospinal fluid test with anti-IgM conjugate.

^cFluorescent treponemal antibody—cerebrospinal fluid test with anti-IgG conjugate.

R = Reactive, NR = Nonreactive, NT = Not tested.

showed a reactivity rate of nearly 80 per cent., which was fairly constant for the several treatment groups.

A summary of the results of the IFA procedures performed on the chimpanzee CSF specimens is given in Table II. The chimpanzee CSF specimens are divided into two groups on the basis of a reactive or non-reactive FTA-ABS test result on a serum sample taken at the time the animal arrived at this laboratory, *i.e.* before any were subjected to experimental infection. After experimental treponemal infection, CSF specimens from all 29 animals became reactive with the FTA-CSF-IgG test; reactivity first appeared from 14 days to 28 months after infection.

Three chimpanzee CSF specimens of the 341 tested demonstrated treponemal IgM antibody when tested by the FTA-CSF-IgM procedure. These three specimens were from two animals with naturally occurring treponemal antibody. One animal (No. 11) was experimentally infected and the other (No. 23) was not.

The results of performing a 'quantitative' test (saline dilutions only) with the anti-IgM conjugate on serum and CSF collected on the same date from these two animals are shown in Table III (opposite).

Discussion

Failure to obtain positive treponemal IgM test results in the two groups of CSF specimens could be related to the following factors:

- (1) An absence of treponemal IgM antibody;
- (2) The presence of antibody at a level below the range of sensitivity of the detection system;
- (3) A masking of the IgM antibody due to anti-IgM binding by an excess of IgG globulin in the CSF.

It is possible that none of the human specimens contained treponemal IgM antibody. Oxelius and others (1969) reported that IgM levels in CSF from individuals with neurosyphilis decreased after treatment; however, it was not shown that the antibody

TABLE III Results of 'quantitative' IFA tests with anti-IgM conjugate

Animal identification	Date	Cerebrospinal fluid	Serum
No. 11 experimentally infected	Nov. 24, 69	Reactive undiluted	Reactive 1,600*
No. 23 not experimentally infected	Jan. 9, 67 Mar. 23, 67	Reactive 10 Reactive undiluted	Reactive 100 Reactive 1,600

*Dilution factor.

was treponemal IgM. This might explain the failure to find treponemal antibody in specimens from twelve adequately treated individuals and possibly in the specimens from an additional nine inadequately treated persons. Seven of the specimens tested, however, were from untreated individuals who exhibited clinical signs of neurosyphilis. Another question would concern the lability of IgM antibody during the period of frozen storage. Most chimpanzee samples, both serum and CSF, had been stored frozen for as long as 8 years, and these same conditions of storage apparently do not eliminate the reactivity of treponemal IgM antibody in serum (Duncan, in preparation).

Link and Muller (1971) stated that the normal concentration of IgM in CSF has not been established, and at present no method is available to detect IgM in unconcentrated CSF. Except for three specimens, therefore, no attempt was made to quantitate the concentration of IgM globulin by IFA or gel diffusion, but we would expect it to be very low in unconcentrated CSF. However, we would expect the high degree of sensitivity of the IFA system to compensate, in part, for the expected low levels of IgM antibody. In serum from chimpanzees, with or without treponemal antibody in their CSF, treponemal IgM antibody was detected in dilutions as high as 1:1,600. If the failure to detect treponemal IgM antibody in the CSF specimens was due to the relative amount present, it must be very low indeed.

Cohen, Norins, and Julian (1967) showed that IgG antibodies in normal serum inhibited IFA reactivity of IgM antibodies against Gram-negative bacteria. Considering the reactivity rates of the two groups of specimens in the IgG procedure, it is possible that this effect was operative in masking the IgM globulin anti-IgM complex on the treponemal antigen. However, it has been shown that treponemal IgM antibody is readily detected in serum from infected chimpanzees by the FTA-ABS IgM technique (Brown, Kuhn, Tolliver, and Norins, 1970).

The data from this study of the chimpanzee CSF specimens must, of course, be considered separately from those of the human material. In the first place the animals do not have clinical manifestations in the

nervous system similar to man and we cannot categorize any of the animals as having neurosyphilis in spite of the fact that the CSF in some of the chimpanzees did exhibit polycytosis, increased CSF protein, and reactivity in the VDRL slide test. Secondly, some of the chimpanzee samples were obtained sequentially over a period of years when the chimpanzees were infected with other pathogenic treponemes, not necessarily *T. pallidum*.

The corresponding serum results from the two FTA-CSF IgM reactive chimpanzees are interesting (Table III). Of fifteen CSF specimens tested, one (from No. 11) demonstrated treponemal IgM antibody, and the corresponding serum diluted in saline was reactive at a 1:1,600 dilution in the FTA-ABS IgM procedure. The undiluted CSF was reactive, but a 1:10 saline dilution was nonreactive. In a gel diffusion method to detect IgM antibody, the 1:800 dilution of serum produced a precipitation line but the undiluted CSF did not. Treponemes had been identified in this animal's CSF by direct FA staining with an anti-*T. pallidum* conjugate, and at that time a sample of CSF caused a syphilitic infection when inoculated into a rabbit.

Two other IgM reactive CSF specimens were from chimpanzee No. 23. The first specimen had a 'spider-web-like' coagulum, and the second did not. The first CSF was reactive in the FTA-CSF IgM test at a 1:10 dilution, the second specimen was reactive only when tested undiluted. The corresponding sera were reactive at dilutions of 1:100 and 1:1,600 respectively. The serum titre increased 16-fold and the CSF titre decreased to one-tenth the initial titre from the first to the second sampling. The first CSF specimen produced a precipitation line in the gel diffusion method, but the second specimen did not. Treponemal IgM antibody was not detected in subsequent specimens of CSF from this experimentally uninfected and untreated animal over a 5-year follow-up period.

From the results of this study it appears that an FTA-CSF IgM procedure on unconcentrated CSF does not offer a practical means of detecting IgM antibody resulting from treponemal infection of the central nervous system.

Summary

The FTA-CSF IgG test was previously shown to be very sensitive in detecting antitreponemal IgG antibody in the cerebrospinal fluid from syphilitic individuals and chimpanzees. In an attempt to increase specificity for the detection of IgM antibody and to obtain an earlier indication of activity in the central nervous system after infection with *Treponema pallidum*, we used a monospecific anti-IgM conjugate as the indicator in the FTA-CSF test. Results obtained on specimens from humans with a diagnosis of neurosyphilis and on specimens from chimpanzees, with or without experimentally-induced treponemal infection, indicate that the FTA-CSF IgM test does not offer a practical means of detecting IgM antibody resulting from treponemal infection of the central nervous system.

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Nouvelles études de l'épreuve de l'anticorps tréponémique fluorescent sur le liquide céphalo-rachidien (FTA-CSF) avec un conjugué anti-IgM monospécifique

SOMMAIRE

Il a été précédemment établi que le test FTA-CSF IgG était très sensible pour mettre en évidence l'anticorps antitreponémique IgG dans le liquide cérébro-spinal provenant d'individus syphilitiques ou de chimpanzés. Pour essayer d'augmenter la spécificité de la détection de l'anticorps IgM et d'obtenir une indication plus précoce d'une réaction active dans le système nerveux central après une infection à *Treponema pallidum*, nous avons utilisé un conjugué anti IgM comme indicateur dans le test FTA-CSF. Les résultats obtenus sur des échantillons provenant d'hommes chez lesquels le diagnostic de neurosyphilis avait été porté, et chez des chimpanzés atteints ou non d'une infection tréponémique expérimentale, indiquent que le test FTA-CSF IgM n'offre pas une possibilité pratique de reconnaître l'anticorps IgM lors de l'infection tréponémique du système nerveux central.