Modified Fluorescent Antibody Technique to Detect Immunoglobulin M Antibodies to *Toxoplasma gondii* in Congenital Infection

P. W. ROBERTSON* AND VERA KERTESZ

Division of Microbiology, The Prince of Wales Hospital, Randwick, New South Wales 2031, Australia

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Using a modified fluorescent antibody technique immunoglobulin M antibodies to *Toxoplasma gondii* were studied in infants' sera. The modified technique involved prolonged incubation of patients' sera with antigen at 4 C.

Infection with *Toxoplasma gondii* is now recognized as one of the most common causes of congenital infection (2). The serological diagnosis of congenital toxoplasma infection is of particular value in that difficulties may be associated with cultivating the causative organism. Intrauterine infection may be established by demonstrating specific immunoglobulin M (IgM) antibodies in cord or neonatal serum (1). This technique has the advantage that only a single specimen of serum is required to establish a diagnosis, whereas if IgG antibodies are examined then it is necessary to carry out repeated tests to distinguish between fetal and maternal antibody. Remington et al. (8) have shown that by using a fluorescent antibody technique it is possible to demonstrate IgM antibodies to *T. gondii* in neonatal and cord serum. Difficulties have been reported with this technique, however, and these have been attributed to the conjugated anti-IgM serum used in the test (4).

The purpose of this brief communication is to report our findings that the binding of IgM antibodies from neonatal serum to the *Toxoplasma* antigen is enhanced by prolonging the incubation time and carrying out the reaction at 4 C.

In an initial attempt to develop the toxoplasma antibody technique in our laboratory, we examined cord serum from an infant in whom prolonged toxoplasma hemagglutination (HA) and complement fixation (CF) (3) antibody levels had been observed during the first year of life. Using three different commercial sources of toxoplasma antigen and three different commercial sources of fluorescein isothiocyanate-labeled anti-human IgM, we were unable to show any fluorescence with this serum using Remington's original method. All attempts using prolonged incubation times, both at 22 and 37 C, were unsuccessful in that

known negative sera showed fluorescence with parasite. When the patient's serum was reacted with the toxoplasma antigen on slides in a moist chamber at 4 C for 18 h, it was possible to achieve a satisfactory result. There was no loss of specificity with this increased incubation, as shown by a complete loss of reactivity when the serum was pretreated with 0.2 M 2-mercaptoethanol. Apart from this increased incubation time, the method used was the same as that reported by Remington et al. (8).

The results with the modified technique were compared with those obtained with Remington's original method using sera from 74 infants under 1 year of age, each of whom presented symptoms suggestive of congenital infection. Five sera were positive by the modified technique when screened at a serum dilution of 1:10, and in each the test was positive when the serum was further diluted to 1:50. With the unmodified IgM fluorescent antibody test, only two of these sera yielded a weakly positive reaction at a 1:10 dilution, and all were negative when the serum was diluted 1:50. In each of the five patients yielding a positive modified test, the diagnosis of congenital infection was supported by increased serum levels of IgM immunoglobulins (1). All of the infants with a positive modified IgM fluorescent antibody test had antibodies detectable by the HA test but the CF test was positive in only three cases (Table 1). These results show, however, that, although specific antibody was detected by the HA technique in all those with positive IgM antibody tests, there were also 50 out of 69 infants with detectable HA antibody in whom IgM antibody was not detected. The CF test also appears unsuitable as a screening test for congenital or neonatal toxoplasma infections in that two sera with IgM antibody gave negative results with the CF test. These results support the findings of Remington et al. (8) that the toxo-

TABLE 1. Antibodies to *T. gondii* in serum from 74 infants under 1 year of age with suspected congenital infection

Determination	Modified toxoplasma IgM fluorescent antibody test	
	Positive titer ≥ 10	Negative titer < 10
Toxoplasma passive HA test		
Titer ≥ 8	5	50
Titer < 8	0	19
Toxoplasma CF test		
Titer ≥ 8	3	10
Titer < 8	2	59

plasma IgM fluorescent antibody technique is the method of choice in the diagnosis of congenital infection with *T. gondii*.

The need for increased antigen-antibody incubation times and lower temperatures in tests designed to detect IgM antibody has been reported in studies with Epstein-Barr virus (6) and in rubella HA inhibition testing (7). These findings support those of the present study in which the binding of toxoplasma-IgM antibody has been shown to require prolonged incubation time at a low temperature. It is possible

that the different behavior if IgM antibody in these systems is related to the low affinity of this class of antibody (5).

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