PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1184–1188 (1975)

Method for Differentiation of Nonspecific from Specfic Toxoplasma IgM Fluorescent Antibodies in Patients with Rheumatoid Factor[1] (38713)

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In 1972, Camargo, Leser, and Rocca (1) reported a high prevalence of toxoplasma IgM fluorescent antibody (IgM-IFA) test titers in sera positive in the latex agglutination test for rheumatoid factor. With the increasing use of the conventional fluorescent antibody (IFA) and IgM-IFA tests in routine diagnostic laboratories, recognition of situations in which false positive results might occur becomes increasingly important. The only other previously reported false positive results in the toxoplasma IgM-IFA test have been in patients with antinuclear antibodies (2). As the IgM-IFA test has proved of value in diagnosing acute congenital and acquired toxoplasmosis (3–6), we considered it important to further evaluate the potential for sera positive in the latex agglutination test to give false positive results in the IgM-IFA test.

Methods. (*a*) *Serum samples.* Sera positive in the latex agglutination test were obtained from 41 patients. Twenty-five had rheumatoid arthritis, three chronic neck pain, two tendonitis, two degenerative joint disease, and one patient each had bursitis, progressive systemic sclerosis, cervical spondylosis, biliary cirrhosis, trigeminal neuralgia, frozen shoulder syndrome, cellulitis of ankle, chronic glomerulonephritis, and synovitis of knees.

Sera negative in the latex agglutination test were from 51 patients with suspected rheumatoid arthritis or other collagen vascular disorders. Sera from 15 adults with acute acquired toxoplasmosis and from 13 newborns and older infants with congenital toxoplasmosis were obtained from ongoing studies (3, 6). The criteria for the diagnosis of acute acquired or congenital toxoplasmosis in these cases were as previously described (3, 6–9).

(*b*) *Serologic tests.* The latex agglutination test for rheumatoid factor (RF) was performed on all sera with the R-A Test supplied by Hyland Laboratories (Costa Mesa, CA). A positive result was considered for any titer $\geq$1:20. The Sabin-Feldman dye test (DT) was performed as described by Frenkel and Jacobs (10) using the micromodification of Desmonts (personal communication, 1971). A positive titer in the DT was any result $\geq$1:4. The IFA and IgM-IFA tests were performed as previously described (8). A positive result in the IFA test was $\geq$1:16 and in the IgM-IFA test $\geq$1:10 in adults and $\geq$1:2 in infants. Those sera giving apparent false positive results in the IgM-IFA test were tested for presence of antinuclear antibody (ANA) (2).

(*c*) *Treatment of sera with heat-aggregated human IgG.* Human IgG (Immunology, Inc., Lombard, IL, Lot No. 399) at a concentration of 10 mg/ml was heated in normal saline at 63° for 10 min. An equal volume of the heat-aggregated IgG was added to each serum. After incubation for 1½ hr at room temperature, the samples were centrifuged at 10,000*g* for 45 min. Supernates were then tested for RF and for toxoplasma antibody in the conventional IFA and IgM-IFA tests.

Results. Of 41 patients' sera which were positive for RF, 11 had positive serology for toxoplasma. Eight (19.5%) were positive in the DT and conventional IFA test (Table I, cases 1–7 and 10). Of these eight, three were positive in the IgM-IFA test in titers of 1:20 (case 2) and 1:40 (cases 5 and 10).

Of the 33 cases positive for RF (the titers were 1:20, one case; 1:40, five cases; 1:80, five cases; 1:160, five cases; 1:320, three

[1] This investigation was supported by grant AI-04717 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

TABLE I. RESULTS IN 11 SERA WITH RHEUMATOID FACTOR AND POSITIVE SEROLOGY FOR TOXOPLASMA.

Patient no.	Underlying disease	Titer[a]				
		Rheumatoid factor	ANA[c]	Toxoplasma serologic test[b]		
				Dye[d]	IFA	IgM-IFA[d]
1	Rheumatoid arthritis	160	Neg	64	16	Neg
2	Rheumatoid arthritis	2,560	Neg	128	256	20
3	Cervical spondylosis	2,560	Neg	2,048	1,024	Neg
4	Rheumatoid arthritis	320	Neg	256	256	Neg
5	Rheumatoid arthritis	5,120	4	2,048	1,024	40
6	Cervical pain, unknown etiology	80	Neg	128	64	Neg
7	Tendonitis of foot	20	Neg	256	256	Neg
8	Probable cervical disc disease	160	Neg	Neg	Neg	10
9	Frozen shoulder syndrome	40	Neg	Neg	Neg	20
10	Rheumatoid arthritis	1,280	Neg	128	1,024	80
11	Rheumatoid arthritis	320	Neg	Neg	Neg	20

[a] Reciprocal.

[b] Dye = Sabin Feldman dye test for toxoplasmosis. IFA = Conventional fluorescent antibody test. IgM-IFA = IgM fluorescent antibody test.

[c] ANA = Antinuclear antibody.

[d] IgM-IFA test titers usually fall to low levels or become negative within a few months of the acute infection but in some cases persist longer; dye test titers may remain at levels of 1:1000 or greater for years.

cases; 1:640, six cases; two cases each had titers of 1:1280, 1:2560, 1:5120 and 1:10,240), and negative in the DT, none was positive in the conventional IFA test. However, three were positive in the IgM-IFA test at titers of 1:10, 1:20, and 1:20 (cases 8, 9, and 11, respectively, Table I). Of the sera positive in the IFA or IgM-IFA test only the serum from case 5 was positive for ANA.

Of the 51 sera from patients with suspected rheumatoid arthritis or other collagen vascular diseases which were negative for RF, 19 (37%) were positive in the DT and conventional IFA test (the DT titers were 1:8, one case; 1:16, three cases; 1:32, one case; 1:128, one case; 1:256, one case; 1:152, seven cases; 1:1024, two cases; 1:2048, two cases; 1:4096, one case). None was positive in the IgM-IFA test.

Sera of 15 adults with acute acquired toxoplasmosis were tested for the presence of RF; none was positive (Table II). Ten of the 15 were previously reported (3). The DT titers in the 15 cases ranged from 1:1024 to 1:65,436 and the IgM-IFA titers from 1:10 to $\geq$1:160. A similar study was performed in sera of 13 infants with congenital toxoplasmosis (Table II). The DT titers in the 13 sera ranged from 1:1024 to 1:65,436 and the IgM-IFA titers from 1:20 to $\geq$1:320. Only two (cases BM and BLM) were positive for RF, both at a titer of 1:320.

The following sera were treated with heat-aggregated IgG: (1) four sera from cases in Table I (cases 2, 5, 10, 11) which were positive for RF and IgM-IFA test antibodies, three of which (cases 2, 10, 11) were considered to give false positive results in the IgM-IFA test; (2) two sera from adults with acute acquired toxoplasmosis (cases KK and AS, Table II) having IgM antibodies but no RF; 3) two sera from cases of congenital toxoplasmosis which were positive for RF and IgM-IFA test antibodies (case BM and BLM, Table II). The results are shown in Table III. The procedure did not reduce the titer of RF significantly. However, the IgM-IFA test results were completely negative after treatment. In contrast, the IgM-IFA test titers in sera from each of the cases of acute acquired and congenital toxoplasmosis were still positive and without significant

TABLE II. RESULTS IN 28 SERA FROM CASES OF ACUTE ACQUIRED AND CONGENITAL TOXOPLASMOSIS.

Patient	Age	Rheumatoid factor	Titer[a] Toxoplasma serologic test[b] Dye	IgM-IFA
Data in adults				
AL	25	Neg	8,192	160
JP	30	Neg	16,384	80
DP	19	Neg	4,096	40
CC	23	Neg	1,024	10
MJ	63	Neg	4,096	40
CS	20	Neg	4,096	40
RK	26	Neg	16,384	80
NA	27	Neg	16,384	40
KO	29	Neg	4,096	80
JF	21	Neg	65,436	160
RC	28	Neg	4,096	≥160
AS	26	Neg	4,096	320
AF	16	Neg	32,768	≥160
PD	27	Neg	8,192	640
KK	30	Neg	4,096	320
Data in infants				
PW	14 mo	Neg	16,384	40
TT	1 mo	Neg	65,436	40
ML	6 mo	Neg	8,192	32
BB	10 mo	Neg	2,048	80
GH	2 mo	Neg	65,536	64
BP	Cord blood	Neg	4,096	20
IB	11 days	Neg	1,024	64
BH	Cord blood	Neg	2,048	80
BK	5 mo	Neg	2,048	80
BM	6 days	320	8,192	320
BLM	9 days	320	32,768	≥320
BL	1 mo	Neg	2,048	40
BLR	13 days	Neg	8,192	≥160

[a] Reciprocal.
[b] See Table I.

change after treatment. Conventional IFA antibody titers in all sera were unaltered by treatment with the heat-aggregated IgG.

Not shown in the tables are results obtained with the treatment procedure, in sera of two infants with congenital syphilis who on follow-up were proved to have maternally transmitted toxoplasma antibodies and not congenital toxoplasmosis. Each had RF and positive DT and IgM-IFA test titers for toxoplasmosis in the newborn period. The IgM-IFA test titers were negative after the treatment procedure.

Discussion. The results described above confirm those reported by Camargo et al., who first reported that false positive results in the toxoplasma IgM-IFA test can occur in sera containing both RF and IgG toxoplasma antibody. However, in the present study, of 33 patients with RF but no detectable toxoplasma antibodies in either the DT or conventional IFA test, three (9.1%) were positive in the IgM-IFA test. The reason for these latter results is unclear. It would seem that the presence of IgG toxoplasma antibodies would be necessary for the false positive results to occur, the patient's IgG antibody combining with antigen on the parasite and this IgG with the same patient's RF (IgM antibody directed against the patient's IgG). When the fluorescein-conjugated anti-IgM is added, it would combine with the patient's RF. Of interest is the fact that, as was observed with serum containing

TABLE III. RESULTS OF TREATMENT OF SERA WITH HEAT-AGGREGATED IgG ON THE IgM-IFA TEST TITER.

		Titer[a]			
			Toxoplasma serologic test[b]		
Category	Patient	Rheumatoid factor	Dye	IgM-IFA before treatment	IgM-IFA after treatment
False positive IgM-IFA[e]	2[c]	2,560	128	20	Neg[d]
	10[c]	1,280	128	80	Neg[d]
	11[c]	320	Neg	20	Neg[d]
Acute acquired toxoplasmosis	5[c]	5,120	2,048	40	40
	As[f]	Neg	4,096	320	320
	KK[f]	Neg	4,096	320	320
Infants with congenital toxoplasmosis	BM[f]	320	8,192	32[g]	32
	BLM[f]	320	32,768	80[g]	80

[a] Reciprocal.
[b] See Table I.
[c] From Table I.
[d] Negative < 2.
[e] See discussion.
[f] From Table II.
[g] Differences in IgM-IFA test titers from those in Table II are due to prolonged storage of the sera from the time of original testing.

ANA (2), not all sera which contained RF gave false positive results in the IgM-IFA test. The reason for this is unclear. There did not appear to be a correlation between titer of RF and level of titer in the IgM-IFA test (e.g., cases 2 and 3 in Table I).

One explanation for our results may be that these sera had ANA but in amounts too small to be detected by the methods employed. As RF can bind IgG-ANA (11) and such ANA may bind toxoplasma organisms (2), this may explain our finding of positive IgM-IFA test results in sera containing RF but no toxoplasma antibodies. In patients' sera containing demonstrable RF and amounts of IgG-ANA too small to detect by our methods, the fluorescein-conjugated anti-IgM would attach to the RF-ANA-toxoplasma complex, giving false positive IgM-IFA test results in the absence of toxoplasma antibody. Another possibility is that IgM-ANA was present in these sera and was not detected, as an IgG-specific fluorescein conjugate is used in the test for ANA. If such were the case, such IgM-ANA combining with toxoplasma organisms would be detected with our fluorescein-conjugated antiserum to IgM. Studies are in progress to better define the mechanisms underlying our observations, by using an insoluble immunoadsorbant with elution of the antiglobulins for subsequent study in both the IgM-IFA and latex agglutination tests.

The IgM-IFA titer of 1:40 in case 5 (Table I) is frequently seen in patients with DT titers of 1:2048 and may reflect either recent infection or prolonged persistence of IgM antibody (3, 9). However, in cases 2 and 10, the DT titer of 1:128 is much lower than that usually observed in the acute infection (3) and follow-up sera in these cases did not reveal a rising DT titer. In the presence of stable DT titers of 1:128, IgM-IFA titers are almost always negative, suggesting that the results in the IgM-IFA test in these two cases represent false positive titers. Although the heat-aggregated IgG employed in these studies did not significantly affect the titer of RF, it did remove toxoplasma IgM-IFA reactivity from sera with what are considered to be false positive results but not from sera of proved cases of acute congenital and

acquired toxoplasmosis. Similar findings were reported by Camargo et al. in serum of adults. Thus, absorption with heat-aggregated IgG appears to selectively remove quantitatively and qualitatively those antibodies detectable in the cross-reactive toxoplasma IgM-IFA test but appears to have little effect on those antibodies (RF) detectable by the more gross latex agglutination test (12). Indeed, difficulty has been encountered in attempts at total absorption of antibodies reactive in the wide variety of tests utilized to detect anti-immunoglobulins (12).

This simple absorption procedure can be utilized when RF is found in adults or infants with suspected acute toxoplasmosis to differentiate an IgM-IFA test result due to RF from that due to toxoplasma-specific IgM antibody.

Summary. In a study performed to define the prevalence of false positive toxoplasma IgM-IFA test results in sera containing RF, 8 (19.5%) of 41 sera which were positive for RF were positive in the toxoplasma DT and conventional toxoplasma IFA test. Three of these eight were also positive in the toxoplasma IgM-IFA test and in two, the results were considered to be false positives. Of the 33 sera remaining which were positive for RF but negative in both the DT and conventional IFA test, three were positive in the toxoplasma IgM-IFA test. Of 51 sera from patients with suspected rheumatoid arthritis or other collagen vascular disorders, all of which were negative when tested for RF, none was positive for toxoplasma IgM antibodies in the IgM-IFA test.

Sera from 15 adults with the acute lymphadenopathic form of toxoplasmosis and 13 infants with congenital toxoplasmosis were tested for the presence of RF. Whereas none of the sera from the acquired cases had demonstrable RF, two of the congenital cases had RF, and their titers were both 1:320.

False positive IgM-IFA test results became negative after treatment of sera with heat-aggregated IgG. In contrast, IgM-IFA test titers in cases of acute congenital or acquired toxoplasmosis were unaffected by this treatment. Thus, treatment with heat-aggregated IgG can be used to differentiate false positive IgM-IFA test titers due to RF from those due to specific IgM toxoplasma antibody.

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Received Sept. 30, 1974. P.S.E.B.M., 1975, Vol. 148.