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DETECTION OF IgM ANTI-TOXOPLASMA ANTIBODIES IN ACUTE ACQUIRED AND CONGENITAL TOXOPLASMOSIS AFTER PROTEIN A TREATMENT OF SERUM

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SUMMARY

Competition from IgG anti-toxoplasma antibodies was shown to be a main factor for false negative tests for IgM antibodies, both in acute acquired and congenital toxoplasmosis. In this way, removal of IgG from serum with the help of **Staphylococcus aureus** Protein A immunosorbent columns or Protein A-rich bacterial suspensions was a successful way to disclose otherwise undetected IgM antibodies. These are simple techniques for practical purposes that should be routinely used as indicated by results so far obtained. After Protein A treatment of samples IgM antibodies could be found not only in a series of cases with a very recent infection, most of which showing high IgG antibody titers, but also in the course of acute infections when IgM tests had become already negative. In several infants clinically suspected of congenital toxoplasmosis, IgM antibodies could be detected only after Protein A absorption of serum samples. Such "blocked" IgM antibodies could be shown by the immunofluorescence test, as well as by the hemagglutination test, the latter performed before and after treatment of samples with 2-mercaptoethanol.

INTRODUCTION

In human toxoplasmosis an important question we expect serology to answer is about the infection actual duration, whetner a recent or an old one. For this purpose, quantitative tests are of limited value since although high titers are usually associated with recent infections, many times they can be observed for months or even years after the acute disease has subsided. Titers are also misleading since recent infections can show as low titers as seen in most reactive, clinically normal people in a population.

The diagnostic value of IgM anti-toxoplasma antibodies in serum was first demonstrated by REMINGTON et al., as a mark of recently acquired infections [10] as well as of congenital toxoplasmosis in the infant [9]. However, false results are not rarely observed in immunofluorescence tests for IgM anti-toxoplasma antibodies. False positives can result from rheumatoid factors, or IgM anti-IgG antibodies in serum, which should be previously removed through either absorption with polymerized IgG or blocking with heat-aggregated gammaglobulin [4]. False negatives have long been recognized, and traced to different causes as a poor reactivity of anti-IgM fluorescent conjugates or competition from IgG antibodies, which thus "block" IgM antibodies.

In this paper we present our evidences of such blocking by high-titered IgG anti-toxoplasma antibodies of IgM antibodies, which usually occur in much lower levels in serum. By removing IgG antibodies with the help of Protein

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A, we could disclose "blocked" IgM antibodies in serum, in cases of recently acquired toxoplasmosis as well as of clinically suspected congenital toxoplasmosis.

MATERIAL AND METHODS

Serum samples — Venous blood serum obtained for serological tests was used the same day or after 1 or 2 days at 4°C.

Tests for toxoplasmosis — A battery of tests was routinely performed including IgG and IgM immunofluorescence (IF), hemagglutination (HA) and complement fixation (CF) tests, as previously described [2]. Specificity of positive IgM-IF tests was always verified by repeating the test after adding to serum heat-aggregated gamma globulin or sorbing with insolubilized IgG [4]. IgM antibodies could also be identified in the hemagglutination test by assaying samples before and after treatment with 2-mercaptoethanol (2 ME) a significant decrease in titer or even negativation of the test being observed [5].

IgG removal from serum — Protein A from **Staphylococcus aureus,** either as an immunosorbent column or as a bacterial cells suspension, was used to remove IgG from serum samples [7]. Efficiency of IgG removal was indicated by negativation of a previously positive IgG-IF test or by a significant reduction in the serum reactivity to just a weakly positive or doubtful result at a 1:16 or 1:64 dilution. To a 4 cm X 0.5 cm Protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) column, 0,5 ml serum was added, followed by PBS. Eluate was collected in 0.5 ml volumes and the two first samples discarded. The third one, corresponding to a 1:2 dilution of serum, was used in the tests. Such dilution was experimentally determined by comparing urea concentrations in sample an eluates. Columns could be repeatedly used for several hundreds of sera, after washing each time with about 3 ml volumes successively of 1 N acetic acid, until draining a pH 3 eluate, and PBS until pH 7.2, since no activity reduction was observed.

Comparable results could be obtained by adding serum to a 10% cell suspension of Protein A good producing **S. aureus** strain, isolated in the laboratory. For this purpose, large bulk cultures of this strain were produced (Dr. E. Leser, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil). After pipetting 0.1 ml serum to 0.7 ml bacterial suspension, the mixture was kept at room temperature for one hour and centrifuged for 10 minutes at 2,000 g. Supernatant corresponding to a 1:8 serum dilution was used in the tests.

RESULTS

As a result of routine Protein A treatment of serum samples from suspected cases both of acute acquired and congenital toxoplasmosis in a busy serological laboratory, it was observed that in many such cases IgM anti toxoplasma antibodies could be detected in serum only after this removal of IgG antibodies. Samples were subject to Protein A treatment everytime a serological pattern suggestive of a recent [1] or a congenital infection [3] was observed from results of a battery of tests including immunofluorescence, hemagglutination and complement fixation tests.

Table I shows test results for a series of patients clinically suspected of acute acquired toxoplasmosis and presenting high IgG-IF and CF titers and low HA titers, as seen in serological pattern I of a recent **T. gondii** infection [1]. Although the IgM-IF test had been negative, in most cases IgM antibodies could be detected through a significant HA titer decrease, or negativation, when treating serum with 2-ME. After Protein A removal of serum IgG, a positive IgM-IF test could be seen in these cases.

A series of similar cases are displayed in Table II, for which IgM anti-toxoplasma antibodies could be demonstrated both with IF and HA tests after sorbing samples with Protein A. A total negativation of the HA test was then observed after treating the sorbed sample with 2 ME. This occurred also not only for pattern I samples but also for samples presenting a transitional serologic pattern II (1), as shown by case 15 in Table II. In this way, an "early" transitional pattern II could be distinguished from a "late" one, the "early" one presenting "blocked" IgM antibodies which could not be found in the "late" transitional pattern, as seen for patients in Table III. This is clearly seen for patient AM, in Table IV which displays serological sequence in cases of acute acquired

T A B L E I

Serologic patterns and IgM-IF test results before and after Protein A treatment of serum in acute acquired toxoplasmosis

Patients	CF	IgG-IF	HA	HA-2ME	IgM-IF	IgM-IF/Prot. A
1. A.C.F.	640	4,000	512	0	0	256
2. M.F.P.	1280	64,000	1,000	0	0	1,000
3. P.H.	1,280	16,000	2,000	1,000	0	64
4. A.M.C.	1,280	16,000	1,000	32	0	4,000
5. M.S.A.	640	8,000	1,000	64	0	64
6. P.C.	1,280	8,000	256	0	0	16
7. V.M.	640	32,000	1,000	1,000	0	64
8. M.R.N.	2,560	64,000	2,000	64	0	1,000
9. B.P.	5,120	16,000	1,000	0	0	4,000
10. R.B.	640	8,000	1,000	128	0	64
11. M.B.	1,280	16,000	1,000	0	0	1,000
12. M.A.P.	2,560	8,000	256	0	0	256
13. M.S.	640	16,000	256	0	0	256
14. C.C.	320	16,000	256	0	16	256
15. M.A.T.	640	8,000	512	0	0	256
16. L.G.	320	8,000	256	0	0	256
17. N.O.	320	4,000	128	0	0	256

T A B L E II

Serologic patterns and IgM-IF and HA test results before and after Protein A treatment of serum in acute acquired toxoplasmosis

Patients	CF	IgG-IF	IgM-IF	HA	HA-2ME	After Protein A		
						IgM-IF	HA	HA-2ME
1. M.R.	2,560	64,000	16	4,000	0	1,000	1,000	0
2. A.F.	640	4,000	0	2,000	0	256	2,000	0
3. N.H.J.	640	8,000	0	1,000	0	4,000	1,000	0
4. W.M.A.	160	1,000	0	128	0	256	128	0
5. 262	320	8,000	0	1,000	256	1,000	256	0
6. J.R.T.S.	2,560	16,000	0	4,000	256	1,000	1,000	0
7. E.M.	640	16,000	0	1,000	512	1,000	512	0
8. A.H.	320	16,000	0	1,000	512	64	1,000	0
9. M.G.	320	4,000	0	2,000	2,000	1,000	256	0
10. C.S.	1,280	64,000	0	2,000	2,000	1,000	512	0
11. S.S.	320	8,000	0	2,000	1,000	256	128	0
12. 31889	320	8,000	0	512	512	256	512	0
13. N.O.G.	640	16,000	0	1,000	1,000	64	1,000	0
14. S.G.S.	320	8,000	0	2,000	1,000	256	128	0
15. 616	320	16,000	0	16,000	8,000	1,000	256	0

toxoplasmosis. An "early" pattern II in the 1 September sample could be distinguished from a "late" one in the 12 November sample.

In infants suspected of congenital toxoplasmosis, Protein A treatment of serum has been of much help to disclose IgM anti toxoplasma antibodies, as indicated in Table V. For this purpose IF and HA tests or both, were seen as useful procedures, furnishing clearcut results.

DISCUSSION

In human toxoplasmosis demonstration of IgM anti-toxoplasma antibodies in serum is of much help for the diagnosis both of acute acquired and congenital forms of the disease. However, the diagnostic value of tests for IgM antibodies can be limited by false results, either positive or negative. The former are usually

TABLE III

Toxoplasmosis tests results before and after Protein A serum treatment in cases presenting a transitional serological pattern II

Patient	CF	IgG-IF	IgM	HA	HA-2ME	After Protein A	
						IgM-IF	HA
VM.	320	16,000	0	4,000	4,000	0	0
R.R.	320	8,000	0	8,000	8,000	0	0
C.B.	640	16,000	0	2,000	2,000	0	0
M.F.	320	8,000	0	8,000	8,000	0	0
F.C.	640	16,000	0	16,000	16,000	0	0
32206	320	4,000	0	4,000	4,000	0	0
33903	640	8,000	0	8,000	8,000	0	0

TABLE IV

Successive serological tests in patients with acute acquired toxoplasmosis

Patient	Date	CF	IgG-IF	IgM-IF	HA	HA-2ME	After Protein A		
							IgM-IF	HA	HA-2ME
A.M.	1 June/79	640	1,000	4,000	2,000	0	—(*)	—	—
	7 July/79	320	16,000	256	128	0	—	—	—
	1 Sept/79	640	32,000	0	8,000	8,000	256	256	0
	12 Nov/79	1,280	64,000	0	32,000	32,000	0	0	—
A.H.	15 Feb/79	1,280	8,000	0	1,000	32	256	—	—
	16 Apr/79	1,280	8,000	0	64	64	64	—	—
	1 Aug/79	640	16,000	0	4,000	4,000	0	—	—
M.A.	14 March/79	640	8,000	0	1,000	0	64	—	—
	17 Apr/79	320	32,000	0	2,000	2,000	0	—	—

(*) Test not done

TABLE V

Detection of "blocked" IgM anti-toxoplasma antibody in infants suspected of congenital toxoplasmosis

Patients	Age	CF	IgG-IF	IgM-IF	HA	HA-2ME	After Protein A		
							IgM-IF	HA	HA-2ME
L.G.	1 mo.	160	32,000	0	512	64	256	—(*)	—
	3 mo.	320	16,000	0	256	128	16	—	—
B.B.	2 mo.	640	16,000	0	256	0	1,000	256	0
A.C.F.	3 mo.	320	32,000	0	512	512	1,000	512	0
	5 mo.	640	32,000	0	1,000	512	64	0	—
	6 mo.	320	8,000	0	2,000	2,000	0	0	—
C.R.R.	1 mo.	320	8,000	0	512	512	512	512	0
A.S.A.	Newborn	160	8,000	0	0	—	64	—	—
D.M.O.	2 wk.	640	32,000	0	4,000	4,000	0	256	0
F.M.(**)	2 mo.	1,280	128,000	0	512	512	256	128	0
	5 mo.	640	64,000	0	1,000	128	0	0	—

(*) Test not done
(**) Probably post natal infection

due to IgM anti-IgG antibodies, which can be easily blocked or removed from serum. Results here presented suggest false negatives to be due largely to competition from IgG antibodies, the removal of which can then disclose the "blocked" IgM antibodies. Affinity to Protein A for most IgG types affords a simple procedure for their removal from serum, which is also practical for routine purposes. Recently, PYNDIAH et al.[8] have described similar results by testing a chromatographically isolated IgM fraction from serum samples.

As indicated by our results, after removing IgG antibodies from serum with the help of Protein A IgM antibodies could be found either in the IF test or through a significant HA titer difference between samples treated and not treated by 2-ME. This was seen in acute, acquired toxoplasmosis showing a serological pattern of a very recent infection, as well as in later serum samples, already displaying a transitional pattern. Curiously, in a few cases for which 2-ME serum treatment did not result in any significative titer differences, after subjecting samples to Protein A such treatment resulted in complete negativation of the HA test, as seen in patients 12 and 13 (Table II) and CRR (Table V). Thus, hemagglutination activities of IgG and IgM antibodies sometimes seem not to add to each other, as in such sera.

The diagnostic help given by a careful serological investigation is shown for example by patients ACF and NO (Table I). Although low titers were seen in IF and HA tests, a high CF titer called our attention, and IgM antibodies were then investigated both by the HA 2ME test and the IgM-IF Protein A test, which then indicated an acute infection pattern. Serological evolution confirmed such diagnosis.

Finding IgM anti-toxoplasma antibodies in serum is decisive for the early diagnosis of congenital toxoplasmosis. However, this has been found in only a limited percentage of cases, of about 50% of the patients [3,6].

Present results are suggestive that most failures could be due to competition from prevailing IgG antibodies and, as indicated in Table V, we have been successful in demonstrating IgM antibodies with the help of Protein A serum absorption in cases the test had been negative. In our experience, in routine toxoplasmosis serology in a busy laboratory association of such simple procedures as staphylococcal absorption, heat-aggregated gamma globulin and 2-mercaptoethanol treatment of samples is feasible, and from it a more complete serological information can result.

RESUMO

Pesquisa de anticorpos IgM anti-toxoplasma após tratamento do soro com proteína A, nas formas aguda e congênita da toxoplasmose

A competição de anticorpos IgG é o principal fator para a ocorrência de resultados negativos falsos nos testes para anticorpos IgM anti-toxoplasma, tanto nas formas agudas da toxoplasmose adquirida como na doença congênita. Desse modo, a remoção prévia das IgG no soro, através de colunas de imuneadsorvente de Proteína A do **Staphylococcus aureus** ou por meio de suspensões bacterianas com Proteína A, representa processo eficiente para tornar possível a demonstração dos anticorpos IgM que, de outro modo, passariam despercebidos. A simplicidade técnica dessa remoção e os resultados obtidos recomendam que seja utilizada de rotina.

Somente após o tratamento dos soros pela Proteína A foi possível demonstrar a presença de anticorpos IgM anti-toxoplasma em casos de infecção recente, a maioria com altos títulos de anticorpos IgG. O mesmo ocorreu no curso de infecções recentes, depois que os testes habituais para anticorpos IgM já haviam se tornado negativos.

Em vários casos de recém-nascidos com suspeita clínica de toxoplasmose, somente foi possível detectar anticorpos IgM anti-toxoplasma no soro após absorção das amostras pela Proteína A.

Nessas várias eventualidades os anticorpos IgM puderam ser evidenciados pelo teste de imunofluorescência com conjugado específico anti-IgM ou pela queda de títulos, ou negativação do teste, quando as amostras foram ensaiadas pelo teste de hemaglutinação, antes e após tratamento com 2-mercaptoetanol.

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