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DIAGNOSTIC INFORMATION FROM SEROLOGICAL TESTS IN HUMAN TOXOPLASMOSIS

III — Hemagglutination Titers Paradoxical Decrease in Acute Infections

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SUMMARY

Recently, successive serologic patterns could be established in human toxoplasmosis, for recent, transitional and ancient infections. An outstanding difference between high immunofluorescence and low hemagglutination titers is one of the characteristics of pattern I of recent infections, a late rise of hemagglutination titers marking transition to pattern II. However, in about 10% of our cases of acute toxoplasmosis, high hemagglutination titers were seen initially, in the first month of disease, but rapidly followed by a striking paradoxical decrease to low values. This preceded the usual late sudden rise to a second peak of high titers. Hemagglutinating antibodies in early and late peaks could be identified as belonging respectively to IgM and IgG classes.

INTRODUCTION

Recently, successive serologic patterns could be defined in toxoplasma infections, from results furnished by a battery of tests including anti-IgG and anti-IgM immunofluorescence (IF-IgG, IF-IgM), hemagglutination (HA) and complement fixation (CF) tests [2,3]. Such patterns correspond respectively to recent, intermediate and ancient infections.

One of the most outstanding characteristics of recent infection pattern I is a marked difference between high IF-IgG and low HA titers. After a few weeks a sudden rise in HA titers then usually occurs, so that in both transitional pattern II and ancient infection pattern III IF-IgG and HA titers show very close titers.

Sometimes we have observed such a titer difference not to occur initially, that is, during the first weeks of clinical disease HA titers showing as high values as IF-IgG. However, a paradoxical decrease in HA titers rapidly followed, from which a more defined pattern I outline resulted.

In this publication we present a few examples of this curious behaviour of hemagglutination titers in recent toxoplasma infections, for which an explanation is sought.

MATERIAL AND METHODS

Patients — The 17 cases here included were selected from more than a hundred patients clinically suspected of acute toxoplasmosis from whom a sorologic study could be done by submitting successive serum samples to a battery of toxoplasmosis tests.

Serum samples and serologic tests — Blood samples were collected and sera kept as previously indicated [3]. Tests were done as described [2]. Specificity of positive IF-IgM tests was always ensured by previously absorbing

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serum samples with polymerized IgG [1,3]. Throughout this study a standard reference serum previously titrated in parallel with the W.H.O. anti-toxoplasma reference serum was always included in the tests. For the IF-IgG test conversion factor from dilution titers to international units was found to vary between 0.15 and 0.20 [5,6].

Treatment of serum samples with 2-mercapto-ethanol — Samples were diluted 1:16 in a 2-ME saline solution (NaCl 0.15M) to a 0.07 M concentration of the reducting agent. After 1 hour in a 37°C water-bath, further doubling dilutions were prepared in saline solution and directly used in the HA test.

RESULTS AND DISCUSSION

Serologic results for 17 patients with acute toxoplasmosis and presenting initial high HA-titers are displayed in Table I. In every case serum samples collected during the first month of disease presented other test results found in pattern I. Thus, IF-IgG test showed high titers, from 1:4,000 to 1:64,000, with only one serum with a 1:1,000 titer. CF-test titers were also high from 1:160 to 1:2,560. IF-IgM test was positive in all cases, with high titers, from 1:256 to 1:16,000. However, in all these patients, which represent about 10% of the acute or recent toxoplasma infection cases we have been able serologically to follow, HA-titers did not differ significantly from respective IF-IgG titers, both tests showing very similar values for a same serum. As previously remarked, in most initial cases of acute toxoplasmosis HA tests show titers that are 16 to 64 times, or more, lower than respective IF-IgG titers [3]. After periods that varied from weeks to a few months, a paradoxical decrease in HA titers was observed for the 17 patients, while same or even increasing IF-IgG titers occurred. In this way, a complete pattern I outline resulted, with marked differences between high IF-IgG and low HA titers.

At the same time a striking titer decrease and even negativation was observed for the IF-IgM test. In a few cases also a significant decrease in CF titers was seen.

Later on, the usual rise in HA titers occurred in most cases, so that a clearcut pattern II could be observed for at least 10 of the patients, from 3 to 12 months after the beginning of the disease (Table I).

Serum samples with "early", and samples with "late" high HA-titers were both submitted to 2-ME treatment and HA tests repeated. It was observed that while no significant differences with former titers were seen for sera presenting serologic pattern II, for pattern I samples high HA-titers were strikingly reduced to very low values or even to negative results (Table II).

This is suggestive of IgM antibodies to be responsible for the first peak and IgG antibodies for the second peak high HA titers.

The large interval of weeks or months between both peaks is really unusual. Perhaps early and late hemagglutination antibodies do not have the same specificities, being directed to different antigenic determinants. A similar observation can be done not infrequently in acute Chagas'disease [4]. High hemagglutination titers are seen early in this infection, for erythrocytes sensitized with polysaccharide fractions of **T. cruzi.** Such titers decrease quickly to low values which then remain at such levels for life. High hemagglutination titers for protein antigens sensitized cells, however, are of late appearance, several weeks or months in general separating peaks detected respectively by each hemagglutination reagent. It has been shown that also for Chagas' disease, the first peak is due to IgM and the second to IgG antibodies [4].

RESUMO

Significado diagnóstico de testes sorológicos na toxoplasmose humana. III — Queda paradoxal de títulos hemaglutinantes em casos de Toxoplasmose aguda

Em publicações anteriores definimos perfis sorológicos próprios de fases sucessivas da infecção toxoplásmica. Uma das características do perfil I, de infecções recentes, é a acentuada diferença entre títulos baixos no teste HA e altos no teste IF-IgG. A ascenção tardia dos anticorpos hemaglutinantes assinala a passagem para o perfil II, transitória, que precede o perfil III de infecção antiga.

Entretanto, em cerca de 10% dos casos de toxoplasmose aguda que pudemos acompanhar

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TABLE I

Results of toxoplasma tests in successive serum samples for 17 acute disease patients presenting a paradoxical decrease of initially high HA titers

Patients	Time of disease (months)	Serologic tests			
		IF-IgG	IF-IgM	CF	HA
1. A.S.F.	1st mo	8,000	8,000	640	8,000
	3 mo	4,000	4,000	160	1,000
	4 mo	4,000	1,000	160	512
	8 mo	4,000	256	160	4,000
	11 mo	8,000	0	320	16,000
2. J.O.G.F.	1st mo	1,000	16,000	160	16,000
	2 mo	4,000	4,000	640	2,000
	4 mo	4,000	1,000	160	1,000
	6 mo	4,000	64	160	512
	7 mo	64,000	0	640	16,000
3. R.B.	1st mo	32,000	1,000	640	8,000
	3 mo	16,000	0	320	2,000
	4 mo	16,000	0	640	16,000
	6 mo	64,000	0	1,280	32,000
4. J.L.S.C.	1st mo	8,000	4,000	640	4,000
	2 mo	16,000	4,000	1,280	2,000
	4 mo	16,000	64	160	256
	6 mo	8,000	0	160	2,000
5. R.N.S.	1st mo	8,000	1,000	160	8,000
	3 mo	8,000	0	160	512
	6 mo	32,000	0	320	32,000
6. L.G.B.	1st mo	8,000	4,000	2,560	4,000
	3 mo	8,000	0	320	1,000
	6 mo	64,000	0	320	8,000
7. H.P.A.	1st mo	8,000	8,000	640	8,000
	3 mo	16,000	0	320	1,000
	5 mo	16,000	0	80	8,000
8. H.H.J.	1st mo	4,000	4,000	320	8,000
	3 mo	8,000	0	320	1,000
	5 mo	64,000	0	640	8,000
9. A.V.B.	1st mo	8,000	4,000	320	8,000
	2 mo	32,000	0	320	1,000
	3 mo	32,000	0	640	8,000
10. F.L.V.	1st mo	4,000	4,000	320	4,000
	2 mo	16,000	1,000	320	2,000
	9 mo	16,000	0	160	16,000
11. J.W.	1st mo	4,000	4,000	640	4,000
	3 mo	4,000	256	320	256
	8 mo	32,000	0	640	32,000
12. M.I.R.V.	1st mo	64,000	256	320	16,000
	8 mo	16,000	0	320	1,000
	12 mo	8,000	0	160	4,000
13. C.D.G.R.	1st mo	8,000	4,000	1,280	16,000
	2 mo	16,000	64	320	2,000
	5 mo	16,000	0	160	4,000
14. P.C.A.	1st mo	8,000	1,000	640	16,000
	2 mo	8,000	256	320	1,000
	3 mo	8,000	0	320	1,000
15. R.P.S.	1st mo	4,000	8,000	2,560	8,000
	2 mo	16,000	1,000	1,280	1,000
16. A.M.A.N.	1st mo	16,000	8,000	1,280	8,000
	3 mo	16,000	1,000	160	512
17. G.C.C.	1st mo	8,000	4,000	320	16,000
	3 mo	8,000	1,000	160	2,000

TABLE II

Toxoplasmosis HA-test titers before and after 2ME serum treatment, in samples presenting different serologic patterns

Patient	Serologic pattern	HA test titers	
		Before 2ME	After 2ME
J.O.G.F.	I (1st)	16,000	0
	I (2nd) (**)	512	32
	II	16,000	16,000
H.P.A.	I	8,000	0
	II	8,000	8,000
R.P.S.	I (1st)	8,000	0
	I (2nd)	1,000	64
C.D.G.R.	I (1st)	16,000	0
	I (2nd)	2,000	0
A.M.A.N.	I (1st)	8,000	0
	I (2nd)	512	256
R.N.S.	I	8,000	0
F.L.V.	I (1st)	4,000	0
	I (2nd)	2,000	0
A.V.B.	I	8,000	0
G.L.M. (*)	I	16,000	0
A.C.Z. (*)	II	8,000	0
I.L.G. (*)	II	2,000	2,000
A.C.A.M. (*)	II	8,000	8,000
A.R.C. (*)	II	16,000	16,000
A.T.N. (*)	II	4,000	4,000
D.P.S.N. (*)	II	8,000	8,000

(*) From another series of cases
(**) Successive samples

sorologicamente, não havia, de início, a diferencial característica do perfil I, pois que os títulos hemaglutinantes se apresentaram tão elevados quanto os fluorescentes. Porém, em todos esses casos mostraram rápida queda paradoxal, delineando-se então um perfil I característico. Posteriormente houve nova ascenção dos títulos hemaglutinantes, como habitual. Identificaram-se como IgM e como IgG os anticorpos hemaglutinantes responsáveis respectivamente pelos títulos altos nas duas eventualidades sucessivas.

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