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IgG RECOGNIZING 21-24 kDa AND 30-33 kDa TACHYZOITE ANTIGENS SHOW MAXIMUM AVIDITY MATURATION DURING NATURAL AND ACCIDENTAL HUMAN TOXOPLASMOSIS

Margarita VILLAVEDRA(1), Julio BATTISTONI(2) & Alberto NIETO(1)

SUMMARY

We describe the avidity maturation of IgGs in human toxoplasmosis using sequential serum samples from accidental and natural infections. In accidental cases, avidity increased continuously throughout infection while naturally infected patients showed a different profile. Twenty-five percent of sera from chronic patients having specific IgM positive results could be appropriately classified using exclusively the avidity test data. To take advantage of the potentiality of this technique, antigens recognized by IgG showing steeper avidity maturation were identified using immunoblot with KSCN elution. Two clusters of antigens, in the ranges of 21-24 kDa and 30-33 kDa, were identified as the ones that fulfill the aforementioned avidity characteristics.

KEYWORDS: *T. gondii*; Avidity; Diagnostic; Acute phase.

INTRODUCTION

Toxoplasmosis is a universally distributed zoonosis produced by the parasite *T. gondii*. The infection by this parasite is common in warm-blooded animals, including man, and it occurs by the ingestion of tissue cysts (in raw meat) or of viable oocysts (in cat feces). The infection in pregnant women can, in turn, produce acute infection of the fetus by tachyzoite (**T**) invasion through the placenta and transmission to embrionary tissue. This can have a broad array of consequences, some of them as critical as abortion and malformation in the newborn. In immunocompromised patients, particularly those with AIDS, the infection cause toxoplasmic encephalitis and death in 10–20 % of the cases.

The injuries caused by this parasite are produced during the acute phase of a prime infection. Therefore, it is crucial to discriminate between acute and chronic disease in order to take the appropriate therapeutic decisions as soon as possible.

Currently the serological diagnosis of acute phase is based on an increase of specific IgG levels, with concomitant presence of specific IgM. Assays based on a single serum sample do not allow a clear discrimination between a recent and a chronic infection. The trend of specific IgM to persist for a long time, even at high levels, has been reported[3,12].

Assays measuring the avidity of IgG against *T. gondii* crude antigen, using a chaotropic compound (like urea or KSCN) as dissociating agent, had been developed to discriminate acute from chronic infection. The technique is based on the evolution of humoral immunoresponse: at early stages of infection, low-avidity Antibodies (**Ab**) are produced while later ones are associated with higher avidity Antibodies[5,9].

In the present work we studied the avidity maturation in two accidental infections and in three natural infections, and compared the avidity values in these cases with two groups of acute and chronic naturally infected patients.

In order to improve the performance of avidity as serological marker to discriminate between acute and chronic infection we tried to identify by KSCN-elution immunoblot the main antigens recognized by antibodies showing the maximum avidity increase from acute to chronic infection.

MATERIALS AND METHODS

Sera

Serum samples from two accidentally infected patients were analyzed. Seven correspond to Case A, infected in the laboratory by accidental spillage in the eyes, with a suspension of more than 10^8 viable RH strain T/mL. This patient showed high fever and adenopathy during approximately one week and was followed up to two years. Ten samples correspond to Case B, infected in the laboratory by accidental needlestick with a similar suspension. This patient was followed up to one year and showed no symptoms.

Fourteen sequential serum samples from three naturally infected patients were included in the present study (cases D, E and I).

(1) Facultad de Química, Cátedra de Inmunología
(2) Facultad de Ciencias. Universidad de la República. Av. Alfredo Navarro 3051, Montevideo, Uruguay.
Correspondence to: M. Villavedra, Cátedra de Inmunología, Facultad de Química, Casilla de Correos 1157, Montevideo, Uruguay. Telephone nr. int + 598 2 487 43 34, Fax nr. int + 598 2 487 43 20. Electronic mail: mvilla@bilbo.edu.uy.

Seventy-eight samples from different patients than the aforementioned were obtained (no more than 2 from each patient) and characterized. Eighteen of them were classified as acute phase sera and sixty as chronic phase sera. Fifteen of the eighteen serum samples (including two samples of Cases F, G and one of Case H) were classified as acute phase sera because they showed a positive titer for specific IgM and a increasing in IgG titers (indirect ELISA or immunofluorescense assay) in a second blood extraction. The remaining three ones correspond to two seroconverted patients and to a pregnant woman with a confirmed congenitally infected fetus. Thirty six of the sixty classified as chronic phase sera showed negative titers of specific IgM while twenty three showed positive titers for IgM but a lower IgG level in the second blood extraction (this group includes second samples of cases C. D and E). The remaining chronic phase sera came from a patient with more than six months of infection.

Two selected samples from sequences of cases A, B, C, D, E, F, G and H were analyzed by immunoblot. Elapsing time between samples were 609 days for Case A; 141 days for Case B; 46 days for Case C; 345 days for Case D; 248 days for Case E; 49 days for Case F; 63 days for Case G and 105 days for Case H.

The Toxo-ISAGA kit (BioMerieux, France) and the Toxo-LBTex kit (LBTex, Uruguay) were used according to manufacturers' instructions for the serological characterization of serum samples.

Parasites

Tachyzoites from *Toxoplasma gondii* (RH strain) were obtained by intra-peritoneal inoculation of adult Swiss mice with a mixture of $14x10^6$ RH T and $11x10^6$ sarcoma cells (TG180, ATCC)[1]. Four days later, parasites were harvested by washing the peritoneal cavity with 5 mL of 20 mM phosphate buffered saline pH 7.2 (**PBS**). The obtained suspension of tachyzoites was washed three times with PBS by centrifugation (3000 x g).

Antigens

Parasite antigens **(Ag)** were prepared from washed tachyzoites as follows: for ELISA the pellet of tachyzoites were resuspended in PBS containing 1% of sodium deoxycholate (Sigma, USA) and incubated first for 1 h at 37 °C and then for 2 hs at 4 °C. The mixture was centrifuged under refrigeration during one hour at 36600 x g. The pellet was discarded, the supernatant filtered through 0.2 µm Millipore filter (Millipore, USA), aliquoted and stored at -70 °C. Protein concentration was determined by the Biuret assay using bovine serum albumin (**BSA**) as standard. For Immunoblot the pellet of tachyzoites was resuspended in SDS-PAGE sample buffer (approximately $5x10^6$ T/mL), boiled for 15 minutes and frozen at -70 °C.

ELISA

100 µL/well of a 5 µg/mL solution of the ELISA Ag in PBS were incubated overnight at room temperature in polystyrene micro-titer ELISA plates (Maxisorp F, NUNC, Denmark) in moist chamber. After discarding the coating solution, 200 µL/well of 1 % BSA in PBS (PBS-BSA) were dispensed and incubated at room temperature for 1 h. Plates were washed three times with 0.05 % Tween 20 in PBS (**PBS-T**). Serum samples diluted in PBS-T with 1% BSA (**PBS-T-BSA**) were dispensed (100 µL/well) and incubated for 2 h in moist chamber at 37 °C. After the supernatants were discarded, plates were washed as described above and 100 µL/well of an appropriate dilution of the corresponding peroxidase (**PO**) Ab conjugate in PBS-T-BSA were incubated overnight at 4 °C in moist chamber. After discarding the supernatants and washing, 200 µL/well of the substrate solution MBTH/DMAB/ H_2O_2 (40 mM of 3-metil-2-benzotiazoline, 0.8 mM of 3-dimetilaminobenzoic acid in buffer phosphate with 0.03% of H_2O_2) were added and the plates incubated for 20 min with shaking. The optical density at 600 nm (A_{600}) was measured in a Titerteck Multiskan Plus (Flow Laboratories, Helsinki, Finland). A pool of sera from acute patients was used as reference in the IgM indirect ELISA. A pool of sixteen sera from naturally infected patients including those showing high, medium and low titers of specific Ig to *T.gondii* was used as laboratory standard for the IgG ELISA. To compare data from different assays, this IgG standard was titrated both by latex agglutination and indirect IgG ELISA against an WHO International standard (total Immunoglobulins). Arbitrary Units (**AU**) were assigned to this pool of sera. For each assay AU and A_{600} data of the reference sera were correlated by linear regression. For analytical consistency the A_{600} value of each sample was converted to AU equivalent to the reference using that regression[10].

Results were expressed as average values of AU/mL followed by the range of variation observed for the different dilutions used. In no case was this range higher than 15% of the mean value.

For IgM detection a PO-conjugated anti- human IgM (µ chain specific, Dako, USA) was used while for IgG detection a PO-conjugate anti-human IgG (γ chain specific, Sigma, USA) was used.

ELISA Avidity Assays

An IgG indirect ELISA method with a KSCN elution step was used[11]. A certain dilution in PBS-T of each serum that in the previous indirect ELISA showed an A_{600} between 0.8 to 1.2 was poured in eight wells. After sample incubation the supernatants were discarded and 100 µL/well of 3.0, 2.5, 2.0, 1.5, 1.0, and 0.5 M solutions of KSCN in PBS-T were added to 6 wells, while PBS-T was added to the other 2 wells. The plates were incubated 15 minutes at RT with gentle shaking.

Avidity Index calculation

The percentage of elution (**% elution**) for each KSCN concentration was defined as the ratio A_{600} (for the given KSCN concentration) / A_{600} (without KSCN treatment). The avidity index (**AI**) was defined as the KSCN molar concentration that eluted 50% of the originally bound Ab. This value was settled for each serum sample from the linear regression Ln (% of elution) vs. molar concentration of KSCN.

Immunoblotting

SDS-PAGE (12% resolving gels) of $5x10^6$ T per lane was carried out under reducing conditions following standard protocols[8] as well as for immunoblotting[13]. After electrophoresis was completed the gel was blotted onto nitro-cellulose **(NC)** (Schleicher and Schuell, Germany) for 70 min at 12 V in a semidry transfer apparatus (LKB, Sweden). When blotting was completed the strips corresponding to the molecular weight standards were removed and stained with Amido Black while the remainder NC paper was blocked with 5% skimmed milk in PBS overnight at 4 °C; washed with PBS 0.1% Tween 20 (**PBS-T 0.1**) and cut in strips. Each serum sample was diluted 1/50 or 1/100 in PBS-T 0.1; 5% skimmed milk, 2% normal goat serum and incubated with the NC strip for two hours at 37 °C with gentle shaking. The strips were washed for 20 minutes three times with PBS-T 0.1. A goat anti-human

IgG (γ chain specific) alkaline phosphatase conjugate (Sigma, USA) was appropriately diluted in PBS-T 0.1, 5% skimmed milk, 2% normal goat serum and incubated with the NC strip overnight at 4 ºC with continuous and gentle shaking. The strips were washed as above, then once with PBS, and finally in 0.1 M carbonate-bicarbonate buffer, 1.0 mM $MgCl_2$, pH 9.8. The substrate solution of NBT/BCIP (0.3 mg/mL Nitro Blue Tetrazolium, 0.15 mg/mL Bromo-4 cloro-3 indol Phosphate in 0.1 M carbonate-bicarbonate buffer, 1.0 mM $MgCl_2$, pH 9.8) was added and developed according to manufacturer´s instructions (Bio Rad, USA). Relative molecular weight estimates were calculated using regression analysis based on the *Rf* of the molecular weight standards in the corresponding NC strips.

Immunoblot avidity assay

For identification those Ag fractions that elicits specific IgG showing steeper avidity maturation, an elution step was performed on one of the NC strips duplicate. After sample incubation, that strip was treated with 1.5 M KSCN in PBS-T (1 mL/strip) during 15 minutes with gentle shacking at room temperature. The working sera dilution for paired samples was selected as the lower one of the earlier serum from patient sequence, which exhibited a clear decrease of intensity in one or more bands after the KSCN elution. After extensive washes, anti human IgG alkaline phosphatase conjugate was incubated as for regular immunoblot, and development was performed as previously described.

Quantitative data from stained NC sheets were obtained by scanning at 550 nm in a Dual Scanning CS 9000 densitometer (Shimatzu, Kyoto, Japan).

The percentage of the decrease in the intensity of each band was calculated using the heights of the corresponding paired peaks in the stained NC strips with and without KSCN treatment.

Statistical analysis

Wilcoxon´s rank test was used to compare AI of acute and chronic patients.

RESULTS

In both cases of accidental infection, Toxo-ISAGA showed high values during all the period tested while the indirect IgM ELISA titers decreased dramatically after the first month of infection. In both cases an AI smaller than 0.8 was observed during the former 2 to 3 months increasing continuously during infection reaching values above 1.0 after 300 days of infection (Fig. 1).

AI maturation was greatly different among naturally infected patients. In two of the cases studied the slopes of the curves were very flat during 200 days after the first extraction. The last extraction for the three cases studied (345, 248 and 105 days post first sample for Cases D, E and I respectively) showed an AI higher than 1.6 (Fig. 2). Analysis of a collection of sera obtained from acute phase naturally infected patients showed AI of 1.0 ± 0.3 (Table 1), while those from chronic patients showed AI of 1.6 ± 0.5 (Table 2). The AI from both sets of patients samples were found to be significantly different (α <0.002) using Wilcoxon's rank test. A cut-off value of 1.6 was defined (arithmetic mean plus two standard deviations of the AI shown by acute phase sera). All the acute phase sera tested showed AI values ≤ 1.6 (Table 1) while

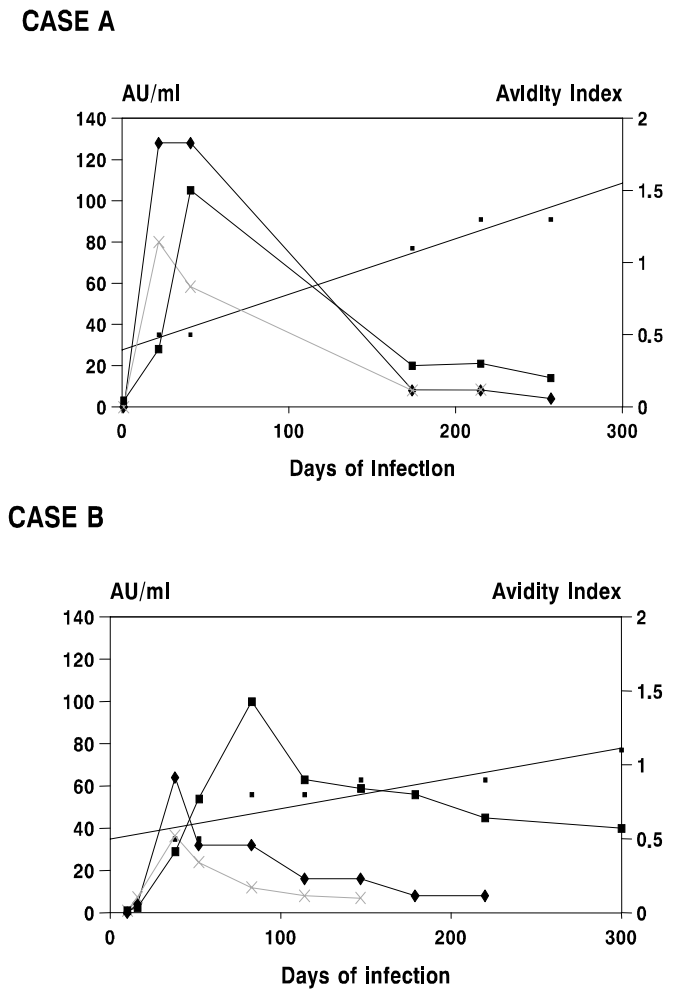

Fig. 1 - *T. gondii* specific antibody responses of two accidentally infected patients (Cases A and B) characterized by latex agglutination (◆); IgM (✕) and IgG (■) by indirect ELISA and Avidity Index of IgG (•) by KSCN elution ELISA method.

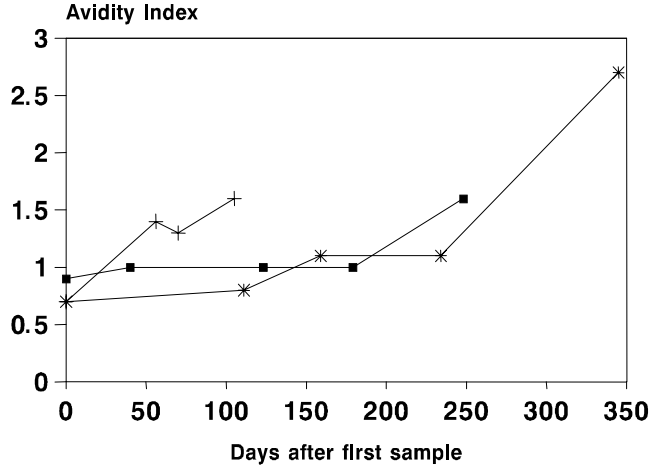

Fig. 2 - AI of sequential serum samples throughout *T. gondii* infection from three naturally infected patients. Case D (✳); Case E (■) and Case I (+).

52% (31/60) of the chronic phase group showed AI ≤ 1.6 (Table 2). In addition, 58% (18/31) of the latter are ISAGA-IgM positive. On the other hand, 25% (6/24) of the ISAGA-IgM positive sera among chronic patients showed AI > 1.6 (Table 2), while 36% (13/36) of the sera from chronic patients which are ISAGA-IgM negative have AI ≤ 1.6 (Table 2). Therefore, the use of AI assay to identify acute phase patients showed 100% sensitivity and 48% specificity.

Two clusters of Ags with molecular weights of 21-24 kDa and 30-33 kDa were identified as the ones against which the increase of IgG avidity was more pronounced from the first to the second sample (Fig. 3 and Fig. 4). IgG recognizing the 21-24 kDa cluster showed a great avidity increase in all tested samples while those recognizing the 30-33 kDa cluster showed the same behavior only in 6 out of 8 (75%) of them (Fig. 3 and Table 3). Other Ags showed avidity increases from acute to chronic infection as well but in smaller percentages of the patients tested (14 to 42%). These Ags showed the following molecular weights: 149, 116, 93, 71, 65-63, 50, 47, 40-34 and 28 kDa (Fig. 3).

In the case of the 21-24 kDa cluster, the percentages of IgG elution with KSCN 1.5 M were in the range of 44 to 100% for the first sample, while for the second sample were in the range of 7 to 61%. In all cases the higher percentage corresponded to the first sample (Table 3).

In the case of the 30-33 kDa band, the percentages of IgG elution were in the range of 25 to 100% for the first sample and from 0 to 47% for the second one (Table 3). In 6 out of 8 (75%) of the cases the percentage of elution for the first sample was higher than that of the second sample.

Table 1
Avidity Index of IgG and serological data of acute phase sera from naturally infected patients

Sera	Avidity Index	Serological data		
		IgM ISAGA	IgG ELISA	Criteria
1	0.4± 0.1	12	48 ± 4	Seroconvertion
2	0.6± 0.1	*nd*	17 ± 2	Increase of IgG
3	0.6± 0.1	*nd*	15 ± 1	Increase of IgG
4	0.6± 0.1	12	71 ± 8	Increase of IgG
5	0.9± 0.1	12	226 ± 8	Increase of IgG
6	0.9± 0.1	12	387 ± 36	Increase of IgG
7	0.9± 0.2	12	1655± 123	Increase of IgG
8	1.0± 0.2	12	54 ± 2	Increase of IgG
9	1.0± 0.2	11	469± 59	Increase of IgG
10	1.0± 0.2	12	569 ± 75	Increase of IgG
11	1.0± 0.2	6	710 ± 41	Increase of IgG
12	1.0± 0.2	12	1210 ± 57	Increase of IgG
13	1.0± 0.1	12	1688 ± 179	Increase of IgG
14	1.1± 0.1	12	580 ± 34	Congenital toxop.
15	1.1± 0.2	12	711 ± 25	Seroconvertion
16	1.2± 0.2	12	412 ± 60	Increase of IgG
17	1.3± 0.2	6	1414 ± 6	Increase of IgG
18	1.6± 0.1	12	3230 ± 470	Increase of IgG
Mean ± SD	1.0 ± 0.3		748 ± 829	

Table 2
Avidity Index of IgG and serological data of chronic phase sera from naturally infected patients

Sera	Avidity Index	Serological data		
		IgM ISAGA	IgG ELISA	Criteria
1	0.8± 0.2	12	211± 23	Decrease of IgG [1]
2	0.8± 0.2	12	1418 ± 40	Decrease of IgG [1]
3	0.9± 0.1	12	143 ± 10	Decrease of IgG [2]
4	0.9± 0.1	-	273 ± 43	IgM (-)
5	1.0± 0.1	11	142 ± 7	Decrease of IgG [1]
6	1.0± 0.2	11	141± 8	Decrease of IgG [1]
7	1.0± 0.2	11	73 ± 5	Decrease of IgG [1]
8	1.0± 0.2	-	160 ± 7	IgM (-)
9	1.0± 0.1	-	48 ± 4	IgM (-)
10	1.0± 0.1	>8	142 ± 7	Decrease of IgG
11	1.0± 0.1	-	123 ± 16	IgM (-) [2]
12	1.1± 0.2	11	151 ± 9	Decrease of IgG [2]
13	1.1± 0.2	9	596 ± 47	Decrease of IgG [2]
14	1.1± 0.2	-	656 ± 29	IgM (-)
15	1.1± 0.2	11	118 ± 17	Decrease of IgG
16	1.1± 0.2	12	850 ± 47	Decrease of IgG [1]
17	1.1± 0.2	12	48 ± 1	Decrease of IgG
18	1.2± 0.1	12	211 ± 28	Decrease of IgG
19	1.2± 0.1	-	167 ± 18	IgM (-)
20	1.2± 0.2	>8	82 ± 7	Decrease of IgG
21	1.3± 0.2	-	80 ± 5	IgM (-)
22	1.4± 0.2	11	883 ± 9	Decrease of IgG
23	1.4± 0.2	-	43 ± 3	IgM (-)
24	1.5± 0.2	-	37 ± 2	IgM (-)
25	1.5± 0.2	-	25 ± 4	IgM (-)
26	1.5± 0.2	>7	117 ± 11	Decrease of IgG
27	1.6± 0.2	-	28 ± 6	IgM (-)
28	1.6± 0.2	-	48 ± 9	IgM (-)
29	1.6± 0.2	-	29 ± 1	IgM (-)
30	1.6± 0.2	11	66 ± 14	Decrease of IgG [2]
31	1.6± 0.2	12	374 ± 17	Decrease of IgG [2]
32	1.7± 0.2	-	145 ± 20	IgM (-)
33	1.7± 0.2	-	161 ± 24	IgM (-)
34	1.7± 0.1	12	563 ± 12	Decrease of IgG
35	1.8± 0.2	-	90 ± 13	IgM (-)
36	1.8± 0.1	-	234 ± 8	IgM (-)
37	1.8± 0.2	-	877 ± 102	IgM (-)
38	1.8± 0.2	-	1780 ± 230	IgM (-)
39	1.8± 0.1	-	135 ± 13	IgM (-) [2]
40	1.9± 0.2	-	117 ± 15	IgM (-)
41	1.9± 0.3	-	69 ± 3	IgM (-)
42	1.9± 0.1	-	2047± 42	IgM (-)
43	1.9± 0.3	-	1950 ± 336	IgM (-)
44	2.0± 0.3	-	60 ± 6	IgM (-)
45	2.0± 0.2	10	62 ± 7	Decrease of IgG
46	2.1± 0.3	-	495± 2	IgM (-)
47	2.1± 0.3	-	22 ± 2	IgM (-)
48	2.1± 0.3	-	3504 ± 374	IgM (-)
49	2.1± 0.2	-	544 ± 74	IgM (-)
50	2.2± 0.3	9	252 ± 6	IgM [2] (-)
51	2.2± 0.3	-	1035 ± 20	
52	2.3± 0.1	9	277 ± 8	Decrease of IgG
53	2.3± 0.3	-	1351 ± 42	IgM (-)
54	2.3± 0.5	10	66 ± 1	Decrease of IgG [2]
55	2.3± 0.3	-	587± 88	IgM (-)
56	2.4± 0.3	-	33 ± 2	IgM (-)
57	2.6± 0.3	10	50 ± 9	Decrease of IgG [2]
58	2.7± 0.1	-	102 ± 13	IgM (-) [2]
59	2.8± 0.2	-	529 ± 10	IgM (-) [2]
60	3.0± 0.4	-	4500 ± 400	IgM (-)
Mean ± SD	1.6 ± 0.5		485 ± 822	

[1] Less than 6 months of infection. [2] More than 6 months of infection.

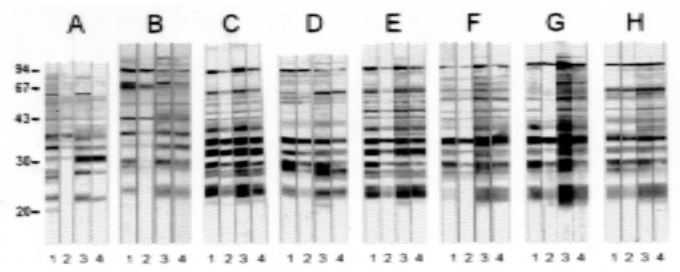

Fig. 3 - Immunoblot analysis of IgG avidity according to elution of specific antibodies observed after treatment of blotted nitrocellulose strips with 1.5 M KSCN in two sequential serum samples from eight *T. gondii* infected patients.
Case A and B - accidentally infected patients. In both patients the first sample was from the acute phase and the second sample from chronic phase.
Cases C to H - naturally infected patients. Cases F, G and H during acute phase. Cases C, D and E during chronic phase.
Lanes 1 and 3- without treatment with KSCN. Lanes 2 and 4 - treated with KSCN.

Table 3
Percentage of antibody elution in two sequential serum samples of *T. gondii* infected patients.

	21-24 kDa		30-33 kDa	
CASES	1st sample	2nd sample	1st sample	2nd sample
A	89	61	48	0
B	100	7	100	41
C	63	33	19	13
D	74	21	65	33
E	76	7	54	0
F	86	24	46	5
G	55	49	39	47
H	44	7	25	32

DISCUSSION

In both cases of accidental infection Toxo-ISAGA test showed high values during all the period tested while indirect IgM ELISA titers decreased dramatically after the first month of infection (Fig. 1). The competition between IgG and IgM for the Ag on the solid phase may explain this fact in the indirect ELISA. Since the concentration of Ab actually decrease along the infection there is an increase of IgG avidity that favors the IgG reaction with Ag on the solid phase in relation to IgM.

The latex agglutination test seems to be better suited to detect the acute phase than the indirect IgM ELISA. IgM and latex agglutination titer maxima are coincident (Fig. 1), due to the greater agglutinating capability of IgM compared with IgG. The decrease in titer observed using the agglutination assay might also be due to the competence for the Ag between IgM and IgG.

Our results confirm that avidity maturation of the IgG response occurs in the majority of the population during *T. gondii* infection (Fig. 1 and

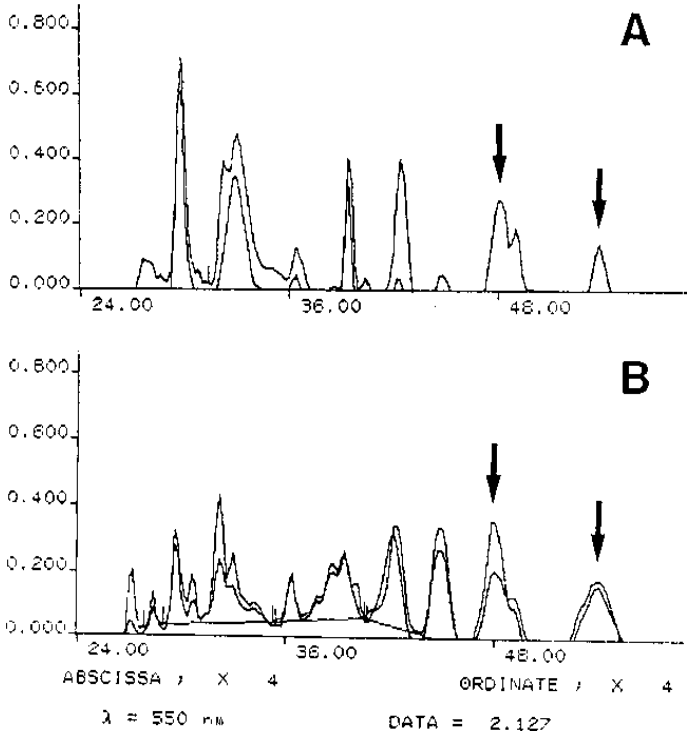

Fig. 4 - Densitometric analysis of nitrocellulose strips from immunoblot corresponding to Case B (panel B, Fig. 3) with and without KSCN elution. Graph A: acute sample; Graph B: chronic sample. Arrows point bands of 21 kDa and 30 kDa respectively.

Fig. 2, Table 1 and Table 2) although big differences could be recorded in their slopes of increase (Fig. 1 and Fig. 2).

To discriminate chronic phase sera using only this criterion, an AI cut-off value of 1.6 (mean plus two standard deviations) was established according to the AI mean values and standard deviations showed by samples from naturally infected patients. The most troublesome cases in the routine toxoplasmosis serology are those patients, which show significant titers of specific IgM during the chronic phase of the infection (24/60 in the population we have tested, Table 2). Our results show that 6 of those 24 cases have AI > 1.6, and therefore 25% of the otherwise doubtful cases (IgM positive) could be classified as chronic, with just one serum sample, through its AI value. We also found that a large fraction of IgM negative chronic patients (36%; 13 out of 36) (Table 2) have AI ≤ 1.6, in contrast with previous results reported by LAPPALAINEN *et al*. who found that all the IgM negative sera (by capture ELISA) have high AI[9]. Therefore we conclude that the AI is not to be used as the only serologic criteria for this aim.

The chronic sera showing an AI < 1.6 with positive IgM correspond, in those cases for which the onset of infection is approximately known, to patients with infection of less than 6 months. Sera from patients with infections of more than 6 months have, in general, IgG of high AI (Table 2). It could be inferred that sera with low AI are probably from acute phase patients or from patients with quite recent infection. On the other hand, the rates of AI increase in the naturally infected patients analyzed show low AI values for a period as long as 200 days (Fig.2). Therefore,

AI values should not be used to estimate time of infection since high AI correlate with chronic infection while low AI do not correlate with time in a defined pattern. This is consistent with previous reports[2,9]. All chronic patients with less than 6 months of infection are IgM positive (by ISAGA) showed an AI ≤ 1.6 while 4 out of 12 patients (33%), with a known period of more than 6 months from the infection time, present AI below 1.6 (Table 2).

We did not find any correlation between AI and titers of specific IgG; although the IgG titers were assessed by ELISA in which the avidity of Ab influences the outcome as well as the actual antibody concentration (Table 1 and Table 2).

In order to identify which Ags are recognized by Abs showing steeper AI increases from acute to chronic infection, KSCN elution from the NC strips were used**.**

This technique has some limitations. In our opinion the most relevant is the selection of sample dilution to be used. An appropriate dilution for a certain Ag could be far from the optimal for another one, against which there are more Abs raised. Similar drawback is foreseen between the first and second sample from a given patient. In order to minimize the incidence of these problems, paired samples from patients at different stages of infection (acute and chronic) with increasing as well as decreasing titers and with different time intervals between samples were tested. Only the Ag bands which showed avidity maturation of specific IgG in all cases (21-24 kDa) or in a large majority (6/8, 30-33 kDa) of the patients were outlined.

Overall the percentage of specific IgG eluted with 1.5 M KSCN is considerably lower in the late sample than in the earlier, although this is particularly so for some Ag clusters. For example in the early sample of Case B, the IgG that reacts with the 21-24 kDa cluster were completely eluted, whereas for the late sample the elution only reached 7% (Table 3). For other bands there was no detectable change in intensity in either of the serum samples. This would imply that there are Ags recognized by IgG showing conventional avidity maturation while for some other Ags, the avidity does not change through the infection. These results suggest that using as immobilized Ags in the ELISA test the purified Ags corresponding to the clusters of 21-24 kDa and 30-33 kDa may optimize the AI assay. Furthermore, some of the patients which do not show a significant AI increase throughout the using a total extract of parasite Ags in the ELISA test, could show avidity maturation of IgG against selected components of the antigen clusters. Also, the analysis of avidity maturation of different IgG subclasses recognizing those identified clusters of Ags may provide some additional information.

Since the dominant Ag SAG-1, which is of wide use even in avidity assays, has been reported to completely lose its reactivity upon reduction[7,14] we conclude that the Ag bands between 30 kDa and 33 kDa, responsible for an steeper avidity maturation do not include SAG-1.

RESUMO

IgG reconhecendo antígenos de taquizoita de 21-24 kDa e 30-33 kDa mostra avidez máxima de maturação na toxoplasmose humana natural e acidental

Descrevemos a avidez de maturação de IgGs na toxoplasmose humana usando amostras sequenciais de soro de infecções naturais e acidentais. Em casos acidentais, a avidez aumentou continuamente através da infeção enquanto os pacientes naturalmente infectados mostraram perfil diferente. 25% dos soros de pacientes crônicos que possuiam resultados positivos de IgM específica puderam ser classificados apropriadamente usando exclusivamente os dados do teste de avidez. Para se aproveitar a potencialidade desta técnica, antígenos reconhecidos pela IgG mostrando progressiva avidez de maturação foram identificados usando-se Imunoblot com eluição com KSCN. Dois grupos de antígenos, com limites de 21-24 kDa e 30-33 kDa, foram identificados como aqueles que preenchem as características de avidez acima mencionadas.

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