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Circulating immune complexes in toxoplasmosis: detection and clinical correlates

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SUMMARY

The ^{125}I-C1q binding test was employed to detect circulating immune complexes in serum of 27 subjects with acute toxoplasmosis. The subjects had no known underlying disease. Elevated C1q binding activity (C1q-BA) was found in the serum of each of three adults with the systemic febrile form of toxoplasmosis, seven of 19 patients with the lymphadenopathic form, and one of four infants with congenital infection. The patients with the systemic form of illness had significantly greater mean C1q-BA than did those with the lymphadenopathic form ($P < 0{\cdot}001$). In six episodes of symptomatic toxoplasmosis associated with elevated C1q-BA, follow-up sera were obtained after resolution of all signs and symptoms. Each of these sera showed normalization of C1q-BA. We conclude that immune complex like material is frequently present in the serum of patients with toxoplasmosis and parallels disease activity.

INTRODUCTION

Circulating immune complexes (CIC) play an important role in many diseases in humans. They can cause damage to kidneys, joints, skin and central nervous system through complement activation with or without local deposition. They can also modulate humoral and cellular immune responses through binding to surface receptors of lymphocytes and phagocytes.

CIC like activity has been reported in sera from patients with a variety of infectious diseases caused by viral, bacterial, protozoal and helminthic agents (Theofilopoulos & Dixon, 1980). However, there has been no report of CIC in patients with toxoplasmosis. Some aspects of toxoplasmosis, notably the occasional occurrence of glomerulonephritis (Ginsburg *et al.*, 1974) or congenital nephrotic syndrome (Shahin, Papadopoulou & Jenis, 1974) suggest a pathogenic role for CIC. Furthermore, the variety of clinical responses to toxoplasma infection in the normal host (e.g., asymptomatic infection, lymphadenopathic disease, febrile illness) might reflect, in part, modulation of host response due to the formation of CIC in some patients. We considered it of interest to determine whether CIC are an important determinant or indicator of the nature of the host response to this infection. For these reasons, we performed experiments to determine if CIC are present in an animal model of toxoplasmosis and in sera of human subjects with various forms of the disease.

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MATERIALS AND METHODS

Rabbits. Two New Zealand White rabbits weighing 2 kg each were obtained from Elkhorn Rabbitry (Watsonville, California, USA). One was injected i.p. with 5×10^4 tachyzoites of the C56 strain of *Toxoplasma gondii*. The day of injection is referred to as day 0. The other rabbit served as a control. From day 7 through day 27 both rabbits received sodium sulphadiazine (Lederle Laboratories, Pearl River, New York, USA) at a concentration of 400 μg/ml in their drinking water to prevent serious illness and death. Serum was collected from both rabbits just prior to infection and at varying intervals thereafter.

Human sera. All sera studied had been received at the Research Institute of the Palo Alto Medical Foundation for diagnostic testing for toxoplasma infection between January, 1980 and January, 1982. Sera were selected for this study if clinical information regarding the patient could be obtained and if the following criteria were met: clinical history, serological studies and histopathological studies when available established a diagnosis of acute toxoplasmosis by published criteria (Remington & McLeod, 1981); no concurrent disease was present; no immunosuppressive therapy had been administered; and an adequate volume of serum was available. All sera had been stored at 4°C for a maximum of 6 days and were subsequently stored at −70°C until used to determine the presence of CIC.

Clinical groups. Subjects with acute toxoplasmosis were divided into three groups on the basis of clinical data. Group 1 consisted of two males and one female with the systemic febrile form of toxoplasmosis. Their ages were 59, 57 and 32 years, respectively. Group 2 consisted of 13 males and six females with the lymphadenopathic form of toxoplasmosis. Their ages ranged from 12 years to 39 years (mean age = 26 years). Group 3 consisted of two male and two female infants with congenital toxoplasmosis. At the time the initial serum was obtained for this study, one of the males was 15 weeks old and the other infants were all in the first day of life.

Two control groups were formed of asymptomatic adults. One group consisted of seven male and four female healthy adult volunteers with a mean age of 32 years. The other group consisted of five male and five female asymptomatic adults with serological evidence of chronic *T. gondii* infection (Remington & McLeod, 1981). Mean age for this group was 37 years. Sera from these individuals were handled as described above for patients' sera.

Serological testing for toxoplasma antibody. All rabbit and human sera were tested for toxoplasma specific antibodies in the Sabin–Feldman dye test (Sabin & Feldman, 1948). Sera from human subjects were also tested for toxoplasma specific IgM antibodies in the double sandwich enzyme linked immunosorbent assay (DS-IgM-ELISA) (Naot & Remington, 1980).

C1q binding test. C1q was obtained from the Blood Research Laboratory (Boston, Massachusetts, USA) and was labelled with ^{125}I using the lactoperoxidase method of Heusser *et al.* (1973). The C1q binding test was performed as described by Zubler & Lambert (1976). Briefly, sera were pre-treated with EDTA, incubated with ^{125}I-C1q, precipitated with polyethylene glycol; and the precipitate was then counted in a gamma counter. In control tubes, an equal amount of ^{125}I-C1q was precipitated with 20% trichloroacetic acid. All test sera were run in duplicate and in parallel with eight or more sera from the control group of healthy volunteers. The result for each tube was calculated as the percentage ^{125}I-C1q precipitated as compared with the trichloroacetic acid control tubes. The mean value of duplicated tests was calculated. For rabbits, the C1q-BA of a serum is expressed as this percentage. For human test sera, the C1q-BA is expressed as standard deviations above the mean C1q-BA for the group of normal control sera which were tested in parallel (Zubler & Lambert, 1976). C1q-BA was considered elevated if it was greater than two standard deviations above the value obtained for the normal sera.

Immunoglobulin levels. IgG antibody and IgM antibody levels were measured on radial immunodiffusion plates (Kallestad Laboratories, Inc., Austin, Texas, USA).

RESULTS

Serological data obtained in the rabbit sera are summarized in Table 1. As can be seen, the C1q-BA

Table 1. C1q binding activity in experimental infection with *T. gondii*

Rabbit	Day	C1q-BA*	Dye test†
Infected	0	7	<4
	3	7	<4
	7	7	<4
	14	21	1,024
	21	29	2,048
	28	25	4,096
	35	22	4,096
	70	5	2,048
Control	0	5	<4
	3	5	<4
	7	6	<4
	10	5	<4
	14	4	<4
	21	4	<4
	70	5	<4

* C1q binding activity expressed as percentage of trichloroacetic acid-precipitable C1q precipitated.

† Expressed as reciprocal of highest positive serum dilution.

value in the infected rabbit rose in parallel with the dye test titre. Subsequently, the C1q-BA values returned to baseline despite persistence of a high dye test titre.

Group 1 patients

Each patient in Group 1 exhibited abnormal C1q-BA.

Case 1.1. A 59 year old Caucasian male was hospitalized with fever to 40°C, chills, diaphoresis, malaise, headache and liver enzymes which were elevated to 1·5–2·0 times the upper limits of normal. His symptoms resolved during therapy with pyrimethamine and sulphadiazine. Subsequently he developed chorioretinitis which responded to a second course of therapy. He has remained well during a 6 month follow-up period.

Case 1.2. A 32 year old Caucasian female was hospitalized with fever to 39°C, chills, malaise, myalgias, headache, anorexia, mild hepatosplenomegaly and diffuse lymphadenopathy. A lymph node obtained at biopsy revealed focal changes consistent with toxoplasmic lymphadenitis (Dorfman & Remington, 1973). Her symptoms resolved spontaneously after 2 months and she has remained well for 4 months.

Case 1.3. A 57 year old Caucasian male presented as an outpatient with fever to 38·1°C, malaise, anorexia and headache; there was no lymphadenopathy or hepatosplenomegaly. Fever and other symptoms resolved gradually over 3 months in the absence of therapy.

Data from these three cases are summarized in Table 2. Cases 1.1 and 1.2 exhibited the highest C1q-BA recorded in human sera in this study. The peak C1q-BA in sera from these two cases were 11 and 23 standard deviations above control values, respectively, whereas the C1q-BA in all the other sera ranged from normal to seven standard deviations above normal. Case 1·3 also had an abnormal C1q-BA of three standard deviations above normal. When compared with Case 1.1 and 1.2, Case 1.3 differed in that fever was lower, hospitalization was not required, no evidence of liver abnormality was found, and the initial serum tested for C1q-BA was sampled later in the course of the illness. In each of the three cases, C1q-BA returned to normal after the patient's clinical recovery. In Case 1.1, C1q-BA rose again when the disease relapsed, and once again returned to normal after clinical recovery.

Table 2. Data in patients with systemic febrile illness due to infection with *T. gondii* (Group 1)

Case	Time from onset of clinical illness (weeks)	Clinical status	C1q-BA*	Serological test titres†	
				Dye test	DS-IgM-ELISA‡
1.1	3	Fever/malaise	11	4,096	65,536
	6	Fever/malaise	7	32,768	65,536
	10	Clinically well	<2	16,384	16,384
	18	Chorioretinitis	5	16,384	4,096
	26	Clinically well	<2	16,384	4,096
1.2	4	Fever/malaise	4	8,192	65,536
	5	Fever/malaise	23	8,192	65,536
	6	Fever/malaise	17	32,768	16,384
	13	Clinically well	<2	8,192	4,096
1.3	11	Fever resolving	3	8,192	16,384
	13	Clinically well	<2	4,096	16,384

* C1q binding activity expressed as standard deviations above mean value of controls.

† Expressed as reciprocal of highest positive serum dilution.

‡ Double sandwich IgM enzyme linked immunosorbent assay.

Table 3. Data in patients with the lymphadenopathic form of toxoplasmosis who had abnormal C1q binding activity (Group 2)

Case	Time from onset of clinical illness (weeks)	C1q-BA*	Serological test titres†	
			Dye test	DS-IgM-ELISA‡
2.1	1	<2	1,024	1,024
	4	7	2,048	2,048
	5	5	2,048	256
	6	4	2,048	64
2.2	1	7	256	16,384
	5	2	2,048	4,096
	10	<2	8,192	1,024
2.3	9	5	8,192	16,384
	11	6	8,192	4,096
2.4	10	3	32,768	4,096
2.5	22	3	4,096	4,096
	28	3	4,096	4,096
2.6	26	3	8,192	<4
	30	3	16,384	<4
	61	<2	8,192	<4
2.7	28	3	8,192	<4

* C1q binding activity expressed as standard deviations above mean value of controls.

† Expressed as reciprocal of highest positive serum dilution

‡ Double sandwich IgM enzyme linked immunosorbent assay.

Table 4. Data in patients with congenital toxoplasmosis (Group 3)

Case	Clinical data	Age (weeks)	C1q-BA*	Serological test titres†	
				Dye test	DS-IgM-ELISA‡
3.1	Male with thalamic calcification and chorioretinitis	15	6	65,538	256
		48	5	8,192	16
3.2	Male with mild hepatosplenomegaly and mild thrombocytopenia	0	<2	8,192	4,096
		3	<2	8,192	1,024
3.3	Female with hydrocephalus; *T. gondii* isolated from autopsy specimens at age 4 weeks	0	<2	4,096	16
		4	<2	4,096	16
3.4	Female with hydrocephalus and chorioretinitis	0	<2	2,048	256
		3	<2	4,096	1,024

* C1q-binding activity expressed as standard deviations above mean value of controls.
† Expressed as reciprocal of highest positive serum dilution.
‡ Double sandwich IgM enzyme linked immunosorbent assay.

Group 2 patients
Of the 19 patients in Group 2, seven (37%) had abnormal C1q-BA. Data obtained in these seven patients are summarized in Table 3. This subgroup of seven patients did not differ significantly from the other 12 in age (mean age 26 years in both subgroups) or sex (six males, one female *vs* seven males, five females). The amount of time which had elapsed between first awareness of lymphadenopathy and sampling of serum for the C1q binding test was not significantly different between the seven patients with abnormal C1q-BA (range: 1–30 weeks, mean: 15 weeks) and the 12 patients with normal C1q-BA (range: 3–43 weeks, mean: 12 weeks).

Total IgG and IgM antibody levels were measured in Group 2 patients to test for a possible influence on our results. For those patients with positive C1q-BA, IgG antibody levels were $12{\cdot}2 \pm 2{\cdot}3$ g/l (mean $\pm$s.d.); while for those with negative C1q-BA, levels were $9{\cdot}4 \pm 3{\cdot}3$ g/l. IgM antibody levels in the two subgroups were $1{\cdot}90 \pm 0{\cdot}60$ g/l and $2{\cdot}26 \pm 1{\cdot}07$ g/l, respectively. Neither of these differences were statistically significant ($P > 0{\cdot}05$, Student's two tailed *t*-test).

When compared to Group 1 patients, Group 2 patients had a lower incidence of abnormal C1q-BA (seven of 19 *vs* three of three, $P = 0{\cdot}08$, Fisher's exact test) and a significantly lower mean peak C1q-BA (two standard deviations above normal *vs* 12 standard deviations above normal, $P < 0{\cdot}001$, Student's two tailed *t*-test). In both Group 1 and Group 2 patients, peak C1q-BA generally occurred at a time when the DS-IgM-ELISA titre was at its peak and often preceded the peak titre in the dye test.

Group 3 patients
One of the four infants with congenital toxoplasmosis had abnormal C1q-BA. Data in these cases are summarized in Table 4.

Adults with chronic symptomatic Toxoplasma gondii *infection*
None of the 10 patients in this group had abnormal C1q-BA.

DISCUSSION

The data presented above reveal that CIC like activity measured by the C1q binding test was present

in a significant proportion of subjects during the acute stage of toxoplasmosis. In the rabbit model, elevation of C1q-BA first appeared in the serum at the same time as did antibody against toxoplasma as measured by the dye test. This is consistent with the hypothesis that the C1q binding test measured immune complexes containing antigen of *T. gondii* and toxoplasma specific antibody.

Our group of patients with the most severe clinical syndrome, i.e., the three patients with the systemic febrile form of toxoplasmosis, all had elevated C1q-BA. In fact, the only two cases (Cases 1.1 and 1.2) in our study which were hospitalized for management of their acute illness (excluding those hospitalized solely for diagnostic lymph node biopsy) were the two cases which exhibited the highest levels of C1q-BA. Additionally, in all three cases, once fever and other symptoms had resolved, C1q-BA returned to the normal range. Thus, the prevalence and degree of CIC in serum of patients with acute toxoplasmosis appeared to correlate with severity of illness. This finding suggests that the presence of CIC during acute toxoplasmosis either may cause a more severe clinical course or may be secondary to some process occurring more actively in the severely ill patients.

The prevalence of abnormal C1q-BA in the group of patients with the lymphadenopathic form of toxoplasmosis was significantly elevated compared to the normal control group but was significantly less than in the group of patients with the systemic, febrile form. Neither age, sex, time from onset of lymphadenopathy, nor mean levels of IgG and IgM antibody differed significantly between the patients with the lymphadenopathic form of toxoplasmosis and normal C1q-BA and those with the lymphadenopathic form and elevated C1q-BA. It would seem important to define the host (e.g., genetic) or parasite factors which account for the presence or absence of C1q-BA in cases of lymphadenopathic toxoplasmosis.

The group of four patients with congenital toxoplasmosis was too small to allow for any conclusions to be made from the data. Possibly of significance is the fact that the only elevated C1q-BA was found in the only subject in this group who was tested at a time beyond the first 4 weeks of life. Further studies in patients with congenital toxoplasmosis are required and the use of age matched controls would be desirable.

A potentially important question not answered by our study relates to the fact that approximately 1% of the population in the USA acquire *T. gondii* infection per year, and the large majority of these individuals remain asymptomatic (Remington & McLeod, 1981). If some of these individuals have CIC, they may account for a significant proportion of otherwise healthy individuals with abnormal C1q-BA and other evidence of CIC. The possible clinicopathological significance of this finding would require further investigation. We are presently attempting to obtain sera from a sufficient population of these individuals for evaluation of the presence of C1q-BA.

When any test is used to measure CIC, the question of the specificity of the test is critical (Agnello, 1981). The ability of C1q to bind to a variety of substances (e.g., endotoxin, DNA, heparin) decreases the specificity of some C1q based tests (Zubler & Lambert, 1976; Lambert *et al.*, 1978). This is far less a problem in the C1q binding test we employed because complexes of C1q and such substances often are not precipitated by polyethylene glycol (Zubler & Lambert, 1976). Thus, in a comparative study of 18 methods for determination of CIC, the C1q binding test results were unaffected by addition of endotoxin to the test mixture and only minimally affected by addition of DNA (Lambert *et al.*, 1978). None of our study patients was receiving heparin. To further characterize the nature of the C1q-BA detected in our patients, we are currently analysing the polyethylene glycol precipitable material for the presence of toxoplasma antigen and anti-toxoplasma antibody. Preliminary data from some of our cases demonstrate that this material is enriched in antibody directed against toxoplasma.

The clinical spectrum of *T. gondii* infection in immunologically normal adults ranges from an asymptomatic infection to a life threatening disease. The factors responsible for determining the response to infection in a given individual have not been identified, nor is it known whether the important determinants are host-dependent, parasite-dependent, or both. Studies of common source outbreaks of *T. gondii* infection have demonstrated that in a given outbreak, some infected individuals develop clinical signs (e.g., lymphadenopathy) while others do not (Teutsch *et al.*, 1979; Luft & Remington, unpublished observations). In such outbreaks, we presume that all infected individuals are infected with the same strain of *T. gondii*. Therefore, host-dependent factors are

likely to be the significant determinants of the clinical response. Because the prevalence and levels of CIC like activity in our study groups correlated with the nature and severity of the illness, we suspect that the presence of CIC is an important indicator and a possible determinant of the nature of the clinical response. Immune complexes are capable of binding to surface receptors on lymphocytes and phagocytes and thereby of regulating the humoral and cellular immune responses (Theofilopoulos, 1980). In so doing, they modify the clinical course of a disease without exhibiting focal pathology due to local tissue deposition. Immune complexes could play such a role in human toxoplasmosis. Hopefully, a better understanding of how the formation and presence of CIC integrate into the overall host response to *T. gondii* infection will further elucidate the significance of CIC in the pathogenesis of toxoplasmosis and the role of the measurement of CIC in the diagnosis and management of infection due to *T. gondii*.

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