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DEMONSTRATION OF ANTIGENIC SIMILARITIES AND VARIATIONS IN EXCRETORY/SECRETORY ANTIGENS OF *TOXOPLASMA GONDII*

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SUMMARY C57BL/6 mice were orally infected with different doses of cysts of ME49 strain of *Toxoplasma gondii* to produce groups of acutely and chronically infected mice. Sera were obtained at different periods post-infection. SDS-PAGE was ran with excretory/secretory antigens of ME49 and RH strains of *T. gondii*, followed by Western blot analyses using the above sera and anti- IgA, IgM, IgG as conjugates. The SDS-PAGE profiles of the two antigens were similar. However the antigenic bands showed variations in all blots, most evidently in IgA blots of chronic sera. IgG blots showed greatest similarities in reactive bands. In IgM blots, more common bands were shown in chronic sera than in acute sera. Variations and similarities in prominence of some bands and time of their appearance were also noted, especially in IgM and IgG blots of chronic sera. Thus antigenic variations and similarities are present in excretory/secretory products of different strains of *T. gondii*.

Toxoplasma gondii is a ubiquitous intracellular protozoan parasite with a world-wide distribution. Infection by this organism are clinically important in certain groups of patients i.e. pregnant women and immunocompromised patients. Research in the quest for antigens of diagnostic significance, especially in predicting period post-infection, are still on-going. Many types of antigens and antigen preparations have been tested in the effort to elucidate the immune responses in toxoplasmosis.

The excretory/secretory antigens (ESA) of *T. gondii* have been demonstrated to elicit a strong and early immune response in infection and immunization (1,2,3,4,5). Van Knapen and Panggabean (6) detected circulating antigen as early as day one post-infection, prior to detection of specific antibody. Hughes and Van Knapen (7) postulated that immediately following entry of *T. gondi* into the host, secreted antigens constitute the major part of the circulating antigen. Further characterization into the nature of this antigen is therefore useful in the development of a potential vaccine using the ESA.

Many strains of *T. gondii* have been reported and therefore it would be important to investigate whether the ESA of different strains will induce similar or different patterns of antigen-antibody interactions. Thus we undertook to analyse Western blots of two different strains of ESA probed with consecutive sera of acutely and chronically-infected mice.

MATERIALS AND METHODS

Infection

Groups of female C57BL/6 mice were orally infected with cysts of ME49 strain of T. gondii (a kind gift from Dr. J. S. Remington, Palo Alto Medical Foundation, USA). To produce acutely infected groups of mice, approximately 50 cysts per mouse were administered while the dose used for establishing chronically infected groups was 5 cysts per mouse. Consecutive sera samples were

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obtained by sacrificing five mice at different periods post-infection (p.i.). For acute sera, they were obtained daily from 1-10 days p.i, while for chronic sera the intervals were every other day for a week, followed by once a week for the next month, then once a forthnight until 82 days p.i. Two batches of each acute and chronic consecutive sera were collected. Acutely infected mice died at approximately 10 days p.i., while chronically infected mice lived until about 3 months p.i.

Antigen preparation

Tachyzoites of the virulent RH strain were available through our routine passage in Swiss albino mice. To obtain tachyzoites of the less virulent ME49 strain, intraperitoneal cysts inoculations were given to cortisone-acetate treated mice. The procedure followed for ESA preparation was derived from the method of Darcy *et al* (1) . Peritoneal washings of twenty mice were pooled and washed twice in RPMI-1640 with penicillin-streptomycin (RPMI-PS). The pellet was then reconstituted in 10ml of RPMI-PS plus 10% fetal calf serum. One ml each was aliquoted into ten tubes and incubated at 37°C for three hours under mild agitation The contents of the tubes were then pooled, pelleted and the supernatant was filtered through 0.45µ membrane filter. The antigen was then aliquoted and stored at -70°C.

SDS-PAGE and Western blots

The ESA was ran on a 12.5% SDS-PAGE according to the method of Laemlii (8), using a Mini-Protein 11 equipment (Biorad, USA). The gels were transferred to nitrocellulose paper, as described by Towbin *et al* (9). The strips of nitrocellulose paper were then probed with the consecutive mice sera at 1:100 dilution for anti-IgG peroxidase conjugate and 1:50 for anti-IgA and anti-IgM conjugates (Sigma,USA). Subsequently, the strips were developed using HRP Color Development (Biorad, USA) and H_2O_2.

RESULTS

Acute infection

In IgA blots (table 1a) only two bands were recognized by each strain of *T. gondii* and one of the bands (36-37 kDa) was shared between the two strains. Two out of seven bands in the IgM blots of ME 49 strain (table 1b) also appeared in IgM blots of RH strain. The bold bands in the IgM blots of ME49 strain were either not prominent or not present in the IgM blots of RH strain. In the IgG blots, all the

Table 1

Molecular Weight Ranges (kDa) Recognized by Sera of Mice Acutely Infected with *Toxoplasma gondii* Strain ME49

Ig Class	IgA		IgM		IgG	
T. gondii Strain	RH	ME49	RH	ME49	RH	ME49
	109		108	145	109 —	105
						**80-64
				45		
				42		
	37 —	36	37 —	35	36 —	37
			31 —	30	30 —	30
				29	29 —	28
				28		
		27				
	(a)		(b)		(c)	

— Shared bands.

** Bold numbers represent prominent bands.

Table 2

Molecular Weight Ranges (kDa) Recognized by Sera of Mice Chronically Infected with *Toxoplasma gondii* Strain ME49

Ig Class	IgA		IgM		IgG	
T. gondii Strain	RH	ME49	RH	ME49	RH	ME49
						****173**
				148		
					118 ▬ **116** (40-82)	(26-82)
			105 ▬ **110**			
			*(7-82)	(12-82)		
		100				
				85-62		
						47
		45				
			43 ▬ **43**		43	
					41	
					39 ▬ **39** (9-82)	(19-82)
38			**37** ▬ 38 (7-82)	(9-26)	**37** ▬ **37**	
					35	
					33 ▬ 33	
			31-30 ▬ 30		**31** ▬ **31** (33-82)	(19-82)
			28 ▬ 28.5 (12-82)	(5-82)	**28** ▬ 29 (26-82)	(12-82)
						26
						13.5
		12				
		(a)		(b)		(c)

▬ Shared bands.

* Number in parentheses indicate time of appearance (days post-infection) of shared bands that occurred or began at different periods post-infection.

** Bold numbers represent prominent bands.

bands of RH strain were also seen in ME49 strain, whereas four (out of five) bands of ME49 strain were noted in RH strain. A broad band of molecular weight range (Mr) 80-64 kDa was clearly seen in IgG blots (table 1c) of ME 49 strain, appearing on day 3 post-infection but this band was not detected in the blots of RH strain. The other bold band of the IgG blots of ME 49 strain was a 37 kDa and it was also seen as a prominent band in IgG blots of RH strain.

Chronic infection

Complete variation in antigenic bands was observed in IgA blots of chronic sera (table 2a). A Mr of 100 kDa was the main band in ME49 strain while Mr 38 kDa was the only band seen in IgA blots of RH strain. IgM blots showed many shared bands between the two strains i.e. Mr 110-105, 43, 38-37, 31-30 and 28 kDa. The Mr 85-62 broad bold IgM band in ME49 strain (also present in ME49 IgG blots of acute infection) was not observed in the same blots of RH strain. It occurred from day 5 post-infection to one month post-infection.. As shown in table 2b, there exist variations in Mr of bold bands and in time of appearance of some shared bands. The latter was exemplified by the 38-37 kDa band which occured at approximately 1-4 weeks post-infection in the ME49 strain but in RH strain the band appeared at approximately 1-12 weeks p.i. In IgG blots there were many common bands between the two strains (six bands). An interesting observation in the IgG blots was that there were more prominent

bands in RH strain than in ME 49 strain. The prominent 173 kDa band in ME 49 strain, however, was not present in RH strain. Variations and similarities in time of appearance of shared bands were also seen in the IgG blots.

DISCUSSION

Studies on the ESA of *T. gondii* have previously shown that they are very immunogenic in human and experimental animals (1,2,3,4,5) . They were also shown to be responsive early in human infection (2) and provided protective immune response in nude rats, thus making them potential candidates for vaccine production (1). The results of these studies have been based on the ESA of the virulent RH strain. The ease of its mass production have made this strain the commonest source of antigen in experimental toxoplasmosis.

Weiss *et al* (10) and Ware and Kasper (11) showed strain-specific antigenic variations when they studied soluble antigens of different strains of *T. gondii*. Studies by Handman *et al* (12) and De La Cruz *et al* (13) however suggested that there may not be significant antigenic differences between different strains of *T. gondii*.

To further elucidate the nature of the humoral responses elicited by the ESA, we undertook to compare antibodies recognition by two different strains of *T. gondii*. In our study, the SDS-PAGE Coomassie blue profiles of the RH and ME49 strains were very similar (figure 1). However the Western blot results clearly showed existence of variations as well as similarities between the recognition of the antibodies of ME 49 -infected mice sera to the ESA of the ME49 and RH strains. The similarities and variations observed can be divided into three categories i.e. Mr of bands, prominence of bands and time of appearance of bands.

Many similar reactive bands were seen in IgG blots of both acute and chronic sera. In contrast IgA blots of chronic sera showed no common bands at all. IgM blots of chronic sera showed more shared bands as compared to IgM blots of acute sera. Variations in time of appearance of common bands are evident in the IgG and IgM blots of chronic sera. In other blots, the common bands occurred during similar time-frame. This aspect may be important since appearance or disappearance of certain bands may help us to determine the approximate time of onset of the infection, a problem crucial in management of pregnant patients. Prominence of bands may reflect the intensity of the humoral response towards the antigenic determinants. . Variations and similarities in Mr of prominent bands were also seen between these two strains, as shown in the IgM and IgG blots of chronic sera.

Our study therefore showed that the choice of the strain of *T. gondii* used for ESA preparation is important in the interpretation and comparison of results of various investigators. An approach that

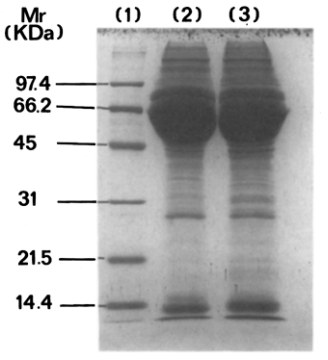

Figure 1. SDS-PAGE Coomassie blue profiles of excretory/secretory antigens of RH strain and ME49 strain. 1, low molecular weight standards; 2, RH strain; 3, ME49 strain.

may be employed in the search for antigens of diagnostic value is the use of a variety of *T..gondii* strains, individually or in combinations

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REFERENCES

(1) Darcy, F., Deslee, D., Santoro, F., Charif, H., Auriault,C., Decoster, A., Duquesne, V. and Capron, A. (1988) Induction of a protective antibody-dependent response against toxoplasmosis by *in vitro* excreted/secreted antigens from tachyzoites of *Toxoplasma gondii*. Parasite Immunology 10: 553-567.

(2) Decoster, A., Darcy, F. and Capron, A. (1988) Recognition of *Toxoplasma gondii* excreted and secreted antigens by human sera from acquired and congenital toxoplasmosis : identification of markers of acute and chronic infection. Clinical and Experimental Immunology 73:376-382.

(3) Charif, H., Darcy, F., Torpier, G., Cesbron-Delauw, M.F. and Capron, A. (1990) *Toxoplasma gondii* : Characterization and Localization of Antigens Secreted from Tachyzoites. Experimental Parasitology 71:114-124.

(4) Darcy, F., Santoro, F., Charif, H., Deslee, D., Hacot, C. and Capron, A (1986) *Toxoplasma gondii* excreted-secreted antigens : first characterization and immunogenicity in humans and experimental infections. Immunobiology 73:241 (abstract).

(5) Darcy, F., Torpier, G., Cesbron-Delauw, M.F., Decoster, A., Ridel, P.R.,Duquesne, V., Charif, H., Godard.,I., Pierce, R.J., Auriault, C. and Capron, A. (1989) *Toxoplasma gondii* : new strategies for the identification of antigens of interest in diagnosis and as potential vaccine. Annals of Biol. clin. 47:451-457.

(6) Knapen, F.V. and Panggabean, S.D. (1977) Detection of circulating antigen during acute infections with *Toxoplasma gondii* by enzyme-linked immunosorbent assay. Journal of Clinical Microbiology 6:545-547.

(7) Hughes, H.P.A. and Knapen, F.V. (1982) Characterization of a secretory antigen from *Toxoplasma gondii* and its role in circulating antigen production. International Journal for Parasitology 12(5):433-437.

(8) Laemlii, U.K. (1970) Ceavage of structural proteins during the assembly of the head of bacteriophage T4. Nature(London) 227:680-685.

(9) Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceedings of the National Academy of Sciences USA 76:4350-4354.

(10) Weiss, L.M., Udem, S.A., Tanowitz, H. and Wittner, M. (1988) Western Blot Analysis of the Antibody Response of Patients with AIDS and Toxoplasma Encephalitis: Antigenic Diversity Among *Toxoplasma* Strains. The Journal of InfectiousDiseases 157(1):7-13.

(11) Ware, P.L. and Kasper, L.H. (1987) Strain-specific antigens of *Toxoplasma gondii*. Infection and Immunity 55(3):778-783.

(12) Handman, E., Goding, J.W. and Remington, J.S. (1980). Detection and characterization of membrane antigens of *Toxoplasma gondii* . Journal of Immunology 124:2578-2583.

(13) De La Cruz, A.A., Dressen, D.W., and Evans, D.L. (1989) Western BlotAnalyses and LD Determinations of *Toxoplasma gondii* Isolates.Veterinary Immunology and Immunopathology 23:355-364.