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SEROLOGICAL ACTIVITY OF A SOLUBLE

THEILERIA CERVI ANTIGEN

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This study was part of a thesis presented to the Department of Veterinary Microbiology, Texas A&M University, in partial fulfillment of the requirements for a Master of Science Degree.

SUMMARY

Two basic cellular erythrocytic antigens were prepared from erythrocytes obtained from a white-tailed deer (Odocoileus virginianus), infected with Theileria cervi. The first antigen was prepared from erythrocytes lysed by freezing, the second from erythrocytes lysed with distilled water. The serologic activity as determined by the complement-fixation (CF) test was greater in the antigen lysed by freezing. Both antigens when solubilized at pH 7.2 using ultrasonic disintegration increased markedly in titer.

The two antigens were pooled and disrupted by ultrasonic disintegration in buffered mediums, ranging from pH 5 to pH 11. Optimal solubilization and serologic activity as measured with the CF test was obtained at pH 11.

The antigen solubilized at pH 11, was used to determine antibody in sera from infected deer by (CF) and by passive hemagglutination (PHA) tests. Both tests resulted in similar but not identical antibody titres.

A gel diffusion test and a ring (interfacial) test, gave no valid results.

INDEX DESCRIPTORS: Theileria cervi effect of soluble antigen on complement-fixation and passive hemagglutination.

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Theileria cervi is a hemoparasite of the white-tailed deer (Odocoileus virginianus), first described by Schaeffler (1961). It was thought by Marburger and Thomas (1965) and Robinson et al (1967) to be a contributing factor of death losses among deer in Texas.

Other organisms of the same genus occur in different parts of the world, where they cause diseases of varying severity in domestic and wild animals.

Laboratory diagnosis of these diseases depends primarily, on microscopic detection of the parasites in stained smears. Serologic tests have been described by Schaeffler (1963), Kuttler and Robinson (1967), Kuttler et al (1967) and Gadir et al (1970). The antigens used in these tests were, for the most part, particulate. In this state, the cell membrane is most responsible for antigenic and serologic activity with the internal structure of the parasite cell not so greatly involved. It is not unreasonable to assume, that the cytoplasm of these parasite cells contains a mosaic of antigens, which when dispersed in molecular phase, i.e., when in solution are capable of more specific or sensitive reactions. Such antigens could find wide use in serologic tests, and possibly prove of greater value in elucidating the antigenic relationship of similar intraerythrocytic parasites.

The purpose of this work therefore, was to investigate the possibility of establishing a method of obtaining a soluble antigen from the erythrocytic stage of T. cervi, that would react with homologous antibody in an in-vitro system.

MATERIALS AND METHODS

Animals

Three deer were splenectomized and infected with *T. cervi* by inoculation of infected blood, one deer was an intact *Theileria* carrier and two were Theileria carriers at the time of splenectomy. They were checked for parasitemia twice a week by examining Giemsa-stained thin blood smears, and used as a source of experimental material.

Isolation of Antigen from Infected Blood

Antigens were prepared from blood of a deer in the acute phase of theileriasis, when a parasitemia of 22% and a packed cell volume of 26% was evident. Approximately 300 ml of blood was collected in equal parts of Alsever's solution and cooled to 4°C. The erythrocytes were separated by centrifugation at 1,500 x g for 30 minutes and washed three times with isotonic 0.15M phosphate buffer solution pH 7.2. (PBS). Each time the buffy coat was removed by aspiration.

The washed erythrocytes were divided into two aliquotes. One aliquote (Antigen A) was lysed by freezing at -20°C overnight and thawed. The second (Antigen B) was lysed by the addition of 10 volumes distilled water. The 2 antigens (sediments) were collected following centrifugation at 34,000 x g for 6 minutes. The 2 antigens were washed with PBS three times by centrifugation at 34,000 x g for 30 minutes, resuspended in equal volumes by PBS and stored at -20°C in 2 ml aliquotes.

Solubilization of Antigens for Serologic Tests

Solubilization in PBS

Aliquotes of Antigen A and B were diluted in PBS to make a 1:20 suspension. Ten ml volumes of each were cooled to 4°C and subjected

to ultrasonic vibration** at 100 watts for 2 minutes. These antigens were then centrifuged at 34,000 x g for 30 minutes and the supernatant fractions retained for serologic testing. These antigens were referred to in Table 1 as Sonicated A and Sonicated B.

Ten ml volumes of the unsonicated suspension of antigens A and B were used for comparison in the same test (Table 1).

For the other serologic tests antigens A and B were pooled and designated antigen AB (pH 7.2).

Solubilization at different H-ion Concentrations

Six aliquotes of 1:20 suspension of antigen AB (pH 7.2) were adjusted to H-ion concentrations ranging from pH 5 to pH 11, using 0.2M potassium hydrogen phthalate ($C_8H_5KO_4$) and 0.2M boric acid (H_3BO_3) buffers as described in the Pharmacopeia of the United States (1960). Antigens at various pH levels were sonicated and centrifuged as described for Sonicated A and B antigens. The supernatant fractions were collected, the pH adjusted to 7.2, and titrated against Theileria positive and negative serum using the CF test (Table 2). These fractions were designated AB(pH 5....pH 11.).

Sonicated and centrifuged antigen AB (pH 11.0) was adjusted to pH 7.2 and used for CF and PHA testing of 34 serums from 6 infected and non-infected deer (Table 4).

Sera

Positive sera were obtained from Theileria infected deer bled at various intervals. Control negative serum was obtained from a deer having no known history of Theileria or other blood parasitic diseases.

** Sonifier Cell Disruptor, Model 185D, Heat Systems, Ultra Sonic, Inc.

Serologic Tests

Complement-fixation Test (CF)

The CF test was performed essentially as described by the United States Department of Agriculture, (1958) with the exception that sera were inactivated by heating at 50°C for 35 minutes in a water-bath, Kuttler et al (1967). A 1:100 antigen dilution was used in 0.5 ml amounts. Serum antibody titers were obtained using serial 2 fold dilutions beginning at 1:5. The end point was the last tube showing complete fixation, by visual observation.

Passive Hemagglutination Test (PHA)

The procedure followed in performing the PHA test has been described by Stavitsky (1954) and Kabat and Mayer (1961). The sera used were inactivated and were the same as those used in the CF test. A 5% solution of antigen AB (pH 11) was used to coat formalized, tanned sheep erythrocytes. The test was conducted in 100 x 12 mm test tubes and the titer recorded was the highest dilution showing a complete agglutination pattern.

Precipitin Tests

Agar Gel Diffusion Test (AGD)

The medium used in the AGD test was 1% special Agar-Noble (Difco) and the test was performed as described by Gourlay and Brocklesby (1967). The dissolved agar was poured in petri-dishes, and a center well plus six circumferential wells 5 mm in diameter were cut. Each circumferential well was 5 mm from the centre well. Various combinations of undiluted positive and negative serum and dissolved antigen AB(pH 11) were matched against one another. The petri-dishes were placed in a moist chamber and inspected at intervals of 6 to 12 hours for 48 hours.

Ring (Interfacial) Test

The ring test was performed using 50 x 5 mm test-tubes and pipettes with capillary tips. Undiluted sera were placed in the bottom of the tubes to a depth of about 10 mm and using AB (ph 11) antigen various dilutions were carefully layered over the sera to a depth of about two times that of the serum. As controls, normal bovine and equine sera and saline were used. The tubes were incubated at 37°C and examined every 30 minutes for 2 hours.

RESULTS

Table 1 compares the CF activity of antigens A and B before and after sonication in PBS. Antigen A showed a higher level of activity. A notable increase in titers occurred in both antigens A and B after sonication.

When antigen AB was suspended in high alkaline buffer mediums (pH 9-11) and sonicated, the suspension became translucent. When the sonicated antigen was centrifuged and smears made of the deposits and examined microscopially, no cellular elements were observed. The components were considered to be in solution.

Table 2 shows the effect of pH on complement-fixing activity of such antigens. A marked increase in CF activity was seen at the higher pH values. Optimum activity was obtained at pH 11. A highly significant correlation coefficient of 0.982 occurs when pH and antigen titers are considered.

Table 3 shows PHA serologic activity of the formalized, tanned sheep erythrocytes treated and untreated with antigen AB (pH 11). The untanned and uncoated erythrocytes failed to show evidence of agglutination in the presence of positive serum, whereas coated, tanned cells showed

typical hemagglutination. Hemagglutination did not occur with negative serum.

Table 4 shows the results of a comparison between the CF and the PHA activity of antigen AB. Both tests were capable of detecting specific serum antibodies, but a significant correlation based on serum titers could not be demonstrated.

TABLE 1

A comparison of the CF activity of Theileria cervi antigens A and B before and after sonication in PBS pH 7.2

TYPE OF ANTIGEN		ANTIGEN DILUTIONS							
		1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
UNSONICATED	A	3+	3+	2+	-	-	-	-	-
SONICATED	A	4+	4+	4+	4+	4+	3+	2+	-
UNSONICATED	B	-	-	-	-	-	-	-	-
SONICATED	B	4+	4+	4+	4+	3+	2+	-	-

Antigen A: lysed by freezing

Antigen B: lysed by distilled water

TABLE 2

Degree of CF activity of *Theileria cervi* antigen AB solubilized by sonication in buffer solutions ranging from pH 5 - 11.

pH	Antigen dilutions							
	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
5	-	-	-	-	-	-	-	-
6	2+	1+	-	-	-	-	-	-
7	3+	2+	1+	-	-	-	-	-
8	4+	3+	2+	1+	1+	-	-	-
9	4+	3+	3+	2+	2+	1+	-	-
10	4+	4+	3+	3+	3+	2+	2+	-
11	4+	4+	4+	3+	3+	3+	2+	-

TABLE 3

Passive Hemagglutination of Sheep erythrocytes treated three different ways in the presence of positive and negative sera.

Positive serum	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Tanned Coated cells	+	+	+	+	±	-	-	-
Tanned uncoated cells	-	-	-	-	-	-	-	-
Untanned coated cells	-	-	-	-	-	-	-	-
Negative serum	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**
Tanned Coated cells	-	-	-	-	-	-	-	-
Tanned uncoated cells	-	-	-	-	-	-	-	-
Untanned coated cells	-	-	-	-	-	-	-	-

TABLE 4

Comparisons of CF and PHA Serum titres and their relation to *Theileria* parasitemia in test deer.

Deer No.	Date	Percent *Theileria* Paraitemia	CF Titre	PHA titre	Remarks
121	7/7/69	0.0	-	-	Splenectomized 23 July, 1969.Carrier at time of Splenectomy
	7/14/69	0.1	-	-	
	8/11/69	2.0	1:10	-	
	8/18/69	12.0	1:320	1:2	
158	3/17/69	0.1	1:160	1:8	Intact Carrier Deer
	6/2/69	1.0	1:160	1:8	
	6/9/69	1.0	1:320	1:32	
	8/4/69	0.5	1:80	1:32	
	8/18/69	0.1	1:80	1:32	
	9/8/69	0.1	1:160	1:32	
159	3/24/69	0.0	-	-	Splenectomized 25 March 1969.Carrier at time of splenectomy
	4/8/69	5.0	1:10	1:2	
	4/21/69	46.0	1:80	1:4	
	4/28/69	1.0	1:80	1:4	
	5/19/69	0.5	1:80	1:4	
	5/26/69	0.2	1:160	1:8	
161	4/8/69	0.0	-	-	Splenectomized April 15, 1969, Infected 25 June, 1969.
	7/7/69	0.2	-	-	
	7/28/69	0.0	-	-	
	8/11/69	0.1	-	-	
	8/28/69	1.0	-	-	
	9/8/69	3.0	1:20	-	
162	4/28/69	0.0	-	-	Splenectomized 25 March, 1969; Infected 2 May, 1969
	5/5/69	0.0	-	-	
	5/21/69	0.1	1:10	-	
	6/2/69	7.4	1:20	1:2	
	6/9/69	42.0	1:160	1:16	
	6/13/69	50.0	1:320	-	
164	1/20/69	0.0	-	-	Splenectomized Infected 20 January, 1969.
	3/27/69	10.0	-	-	
	5/12/69	18.0	1:40	1:8	
	5/27/69	6.0	1:80	1:8	
	7/14/69	4.0	1:160	1:8	
	7/28/69	2.5	1:320	1:8	

The soluble antigen AB produced no precipitin lines on agar gel plates. The results of the ring test were discounted, as the antigen formed a ring at the interface with any and every serum used, including bovine and equine sera.

DISCUSSION

Two batches of T. cervi antigen, obtained from the erythrocytes of an infected deer, using two different methods of lysing, did not fix complement to the same extent. Antigen A, obtained from erythrocytes lysed by freezing, had a higher titer than antigen B, obtained when the erythrocytes were lyzed by water. The difference was not great, and it was unlikely that the method of lysis altered the antigenic properties of either. The variation in the stroma content, could cause a difference in the percentage of active antigen contained in the antigen-stroma desposits after centrifugation.

A notable rise in titer resulted, when the two antigens were sonicated for 2 minutes at 100 watts in a pH 7.2 buffered medium then centrifuged before testing. As with the unsonicated antigen, the titer was higher for antigen A than B. An increase in antigenic activity apparently resulted from dispersion of the cellular components. This increase was in direct proportion to the rise in the pH of the buffer used. At pH 5 the supernatant fraction showed no CF activity at the 1:40 dilution, while at pH 11 complete fixation occurred at 1:160 antigen dilution, with a 3+ reaction at 1:1280. Solubility was greatest at the higher pH values.

The PHA test depended upon adsorption of a soluble antigen on formalized tanned sheep erythrocytes, and subsequent erythrocytic agglutination in the presence of homologous antibodies.

REFERENCES

GADIR, F.A., HIDALGO, R.J. AND KUTTLER, K.L. 1970 - Complement-fixation Antigen Production for Theileria in white-tailed deer (Odocoileous virginianus)
American Journal of Veterinary Research 31: 879-885 .

GOURLAY, R.M. and BROCKLESBY, D.W. 1967 - Preliminary experiments with antigens of T. parva.
British Veterinary Journal, 123: 533-539.

KABAT, E.A. and MAYER, M.M. 1961 - Experimental Immunochemistry. Springfield, Illinois Charles C. Thomas, 119-120.

KUTTLER, K.L. and ROBINSON, R.M. 1967. - A capillary-tube agglutination test forthe detection of Theileria infection in White-tailed deer (Odocoileus virginianus)
Southwestern Veterinarian Vol XXI No. 1, 51-55.

KUTTLER, K.L., ROBINSON, R.M., and ROGERS, W.P 1967 - The Detection of Specific Complement-fixing Antibodies in Serum of White-tailed deer (Odocoileus virginianus) with Theileria infection.
Canadian Journal of Comparative Medicine and Veterinary Science. 31, 354-357.

MARBURGER, R.G. and THOMAS, J.W., 1965 - A die-off in White-tailed deer of the Central Mineral Region of Texas.
Journal of Wildlife Management 29(4): 706-716.

PHARMACOPEIA OF THE UNITED STATES, 1960 - 17th Revision, pp 928-930.

ROBINSON, R.M., KUTTLER K.L., THOMAS, J.W. and MARBURGER, R.G., 1967. Theileriasis in Texas White-tailed deer.
Journal of Wildlife Management, Vol. 31: No. 3, 455-459.

SCHAEFFLER, W.F. 1961. Theileria in White-tailed deer in the UnitedStates
Journal of Protozoology, 8(Supplement): 10, Abstract 30.

SCHAEFFLER,W.F. 1963. - Serological Tests of Theileria cervi in White-tailed deer and other species of Theileria in cattle and sheep.
American Journal of Veterinary Research, 24: 784-791.

STAVITSKY, A.B. 1954, Passive hemagglutination
Journal of Immunology, 72: 360-367.

U.S. DEPARTMENT OF AGRICULTURE 1958 - A manual for conducting the Complement-fixation test for Anaplasmosis
Washington D.C. Government Printing Office.