Blocking Antibodies in Rabbits Infected with *Brucella melitensis.** (24965)

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Blocking antibodies are thermostable antibodies which combine with their specific antigens without visible evidence of agglutination or precipitation. The blocking phenomenon has been observed in insulin resistance, hemolysins, diphtheria toxoid immunization, large injections of pollens and other protein extracts, and the Rh antigen-antibody system. Blocking antibodies also have been reported in association with influenza virus infections and in brucellosis(1). We wish to report a preliminary study on production of blocking antibodies in a small series of rabbits infected with a massive dose of *Brucella melitensis* and the suppressive effect of early treatment with antibiotics.

Method. 1. After blood was obtained for control studies, 6 two-month-old male albino rabbits received *via* ear vein 2 ml of heavy suspension of *Br. melitensis,* strain 363 (courtesy of Dr. W. W. Spink, Univ. of Minnesota Hospitals), grown aerobically 48 hours on tryptose agar. Viable counts of infecting suspension approximated 110 x 10^{14} organisms/ml. Approximately 12 hours after inoculation, 3 of the 6 rabbits were treated with 500 mg streptomycin and 60 mg tetracycline, administered subcutaneously every day for 14 days. The remaining 3 rabbits were left untreated. 2. Blood was obtained at appropriate intervals from all 6 animals *via* ear vein by insertion of a disposable 20 gauge needle. Titers for agglutinating and blocking antibodies, electrophoretic separation of serum proteins and culture for Brucella organisms were obtained from each blood specimen. Agglutinin titer was determined by modification of method of Fitch, Donham, Bishop and Boyd(2). Serum was diluted 2-fold serially from 1:10 to 1:5,120 with 0.85% saline solution. *Br. abortus* antigen (0.5 ml) was added to 0.5 ml of saline (control) and 0.5 ml of each dilution of serum in separate tubes. Final dilution of serum was carried thus to 1:10,240. The antigen was prepared by diluting heavy suspension of heat-killed *Br. abortus* cells to 1:100 with saline solution. Stock antigen suspension was obtained from Agri. Research Service, Animal Disease Eradication Branch, U.S. Dept. of Agriculture. After addition of antigen, the content of each tube was mixed by shaking, then incubated for 48 hours in water bath at 37°C. The tubes were then refrigerated at 5°C for 1 hour. They were examined immediately against a black background with mild agitation. Degree of agglutination was recorded as 0, ±, 1+, 2+, 3+, and 4+, depending on size and number of aggregates as well as degree of clearing of supernate. Agglutinin titer was taken to be the highest dilution of serum showing 1+ or greater agglutination. The blocking test was carried out in same manner as for agglutinin titer, except that 0.03 ml of rabbit anti-brucella serum (agglutinin titer of 1:320) was added to each tube and a saline control tube was included, containing no blocking serum. Blocking was indicated by less agglutination than that in control tube. 3. Electrophoretic studies were carried out in electrophoretic cell (Spinco) using 0.087 M. veronal buffer at pH 8.6. Twenty cu mu of serum was allowed to migrate 16 hours on Whatman No. 3 filter paper. The strips were stained with bromphenol blue dye dissolved in alcohol containing 1% mercuric chloride and then evaluated with Photovolt densitometer (red filter). The areas under individual protein peaks were determined by planimetry. 4. In absorption studies, sera were absorbed twice with heat-killed *Br. abortus* antigen. Each absorption was carried out in water bath at 37°C for 48 hours. 5. Autopsies were performed on all rabbits. Liver and splenic tissues were obtained for histological and cultural studies.

* Supported by U. S. P. H. S. Grant.

† Sincere appreciation is extended to Janet Johnson for technical assistance.

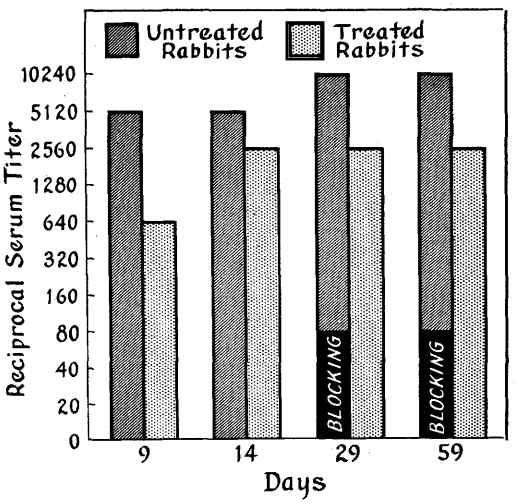

FIG. 1. Antibody titers in sera of rabbits infected with *Br. melitensis*. Blocking antibodies appeared in sera of untreated animals. Prompt treatment with antibiotics seemed to prevent their appearance.

The tissue was prepared for 3 minutes in 50 ml Servall tissue grinder. Aliquot amounts of resulting suspension were cultured on tryptose agar, so that a rough comparison of number of organisms was obtained.

Results. Blood cultures. All control blood cultures were sterile on tryptose agar plates. All infected animals had positive blood cultures within 24 hours after inoculation. In spite of what was considered adequate antibiotic therapy, all treated group of rabbits had persistent Brucella bacteremia for 5 days after initiation of treatment. The untreated group of rabbits, however, suffered persistent Brucella bacteremia for 12 days. One rabbit from untreated group died on 14th day.

Agglutination and blocking antibody studies. Serological studies for agglutinating and blocking antibodies were negative in control sera. Treated rabbits developed a slightly lower agglutinating antibody titer than untreated animals (Fig. 1). Treated animals failed to develop blocking titers throughout the study (59 days), whereas untreated animals (2 surviving) developed a blocking titer of 1:40 and 1:80 respectively by 29th day; this persisted through 59th day without change.

Electrophoretic studies indicated that rabbits left untreated showed a large gamma globulin peak in their sera from 9th day of infection. Gamma globulin levels had increased 2- to 3-fold over those in normal rabbits. Gamma globulin could not be removed by absorption with *Br. abortus* antigen. The treated Brucella-infected animals maintained a relatively normal electrophoretic pattern in their serum during the experiment (Fig. 2, Table I).

Necropsy findings. All untreated animals exhibited a markedly enlarged liver of normal color and consistency. Two had multiple liver abscesses containing greenish-white caseous material; abscesses varied in size from pinpoint to 0.5 cm in diameter. Spleens appeared normal in size and color. No abscesses were found in these organs. Other organs were normal.

Treated animals presented no grossly abnormal findings except for slightly enlarged livers.

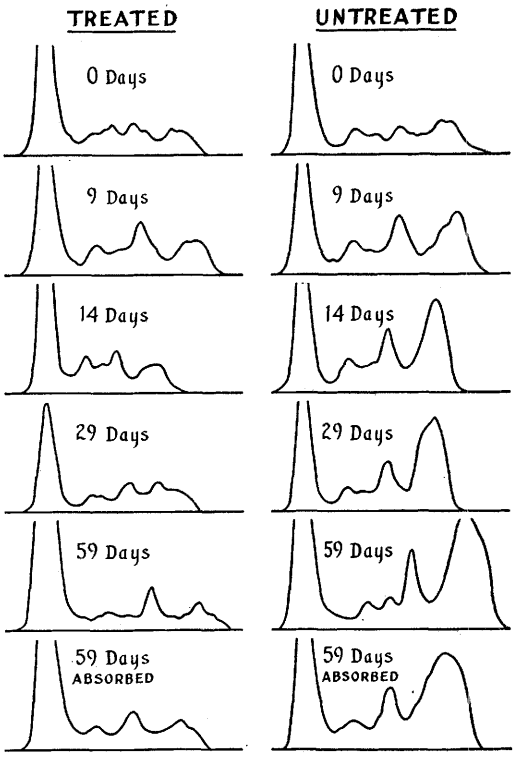

FIG. 2. Electrophoretic patterns of serum proteins of rabbits infected with *Br. melitensis*. Animals treated promptly with streptomycin and tetracycline had normal patterns; those untreated showed a 2-3 fold increase in gamma globulin peak.

TABLE I. Distribution of Serum Proteins in Rabbits Experimentally Infected with *Br. melitensis*. Three animals were treated promptly with a combination of streptomycin and tetracycline, 3 not treated.

Rabbits	Days after infection	Albumin: % of total proteins	Globulins: % of total proteins			
			α-1	α-2	β	γ
Control	0	57.05	4.34	9.83	12.50	16.28
Treated	9	48.21	2.94	9.73	22.88	16.23
	29	53.33	7.19	5.93	15.01	18.54
	59	73.94	3.04	5.17	10.05	7.34
Untreated	9	32.49	2.28	9.91	19.07	35.25
	29	40.09	6.04	4.63	14.78	34.48
	59	45.50	3.50	5.99	13.16	31.92
Absorbed	59					
Treated		63.01	4.02	6.93	13.67	12.37
Untreated		33.80	3.94	9.12	14.56	35.59

Microscopic examination of livers and spleens revealed epithelioid granulomata in untreated animals, compatible with Brucella infections. There also were liver infections with Coccidia. Splenic tissues appeared normal except for one, which showed an abundant proliferation of reticulum with some plasma cells and eosinophils scattered about. Tissues of treated rabbits revealed normal spleens and livers except for one section of liver that showed a possible healing granuloma.

Culture of pooled ground liver and spleen revealed massive Brucella infection only in the liver of untreated animals; only a few colonies of Brucella were cultured from spleens of untreated animals. Cultures from spleens and livers of treated animals yielded only a few colonies of Brucella.

Discussion. These preliminary studies indicated that rabbits infected with large numbers of *Br. melitensis* produced blocking antibodies in low titers by 29th day of infection and persisting through 59th day of observation. In addition, gamma globulins of serum increased 2 to 3 times the normal amount. Absorption of sera with Brucella failed to decrease gamma globulin peak. Rabbits which received antibiotic therapy developed neither increase of gamma globulins nor blocking antibodies.

It appeared that the blocking phenomenon in rabbits infected with Brucella was not correlated with presence of active infection itself, since viable organisms were cultured from both treated and untreated animals; rather, it seems that severity of infection or total amount of antigen in contact with antibody-producing cells might be the deciding factor inducing appearance of the blocking phenomenon. The smaller the amount of active antigen persisting in the animal, the longer it might take to bring about the blocking phenomenon.

This apparent relationship is not unique in brucellosis. Mothers carrying Rh-positive fetuses are known to demonstrate agglutinating anti-Rh antibodies initially, but at a later stage of pregnancy or with subsequent pregnancies by the same father, only incomplete (blocking) antibodies may be demonstrable (3). Experimentally, blocking antibodies induced with purified proteins may appear only after repeated injections with the same antigen(4).

Brahic and others performed experiments similar to ours, inoculating rabbits with *Br. suis*. Using the Coombs test, they were able to demonstrate incomplete antibodies as early as 5 days after infection and lasting more than 60 days(5). Our inability to confirm the early appearance of incomplete (blocking) antibody may depend on the large dose of micro-organisms given, which was about 10^6 larger than that used by Brahic. Furthermore, there was a difference in the test used to demonstrate non-precipitating antibody. Cruickshank infected guinea pigs with *Br. abortus* and concluded as we have, that incomplete antibodies are elaborated only in

later stages of infection(6).

The marked increase in serum gamma globulins following untreated Brucella infection has previously been demonstrated experimentally in guinea pigs(6,7) and in man(8,9). Our absorption studies indicate that this gamma globulin is not absorbed by *Br. abortus* cells. This may be indicative of a nonspecific outpouring of proteins in response to infection. There is a possibility, however, that the acute phase gamma globulins contain some nonabsorbable antibody.

Summary. Six rabbits were infected intravenously with a single massive dose of *Br. melitensis*. Three were treated by subcutaneous administration of streptomycin and tetracycline for 2 weeks and 3 were left untreated. The untreated rabbits developed a markedly increased gamma globulin content in their serum, which was not appreciably changed by absorption with killed whole *Br. abortus* cells. In addition, the blocking phenomenon was demonstrated in low titers in serum of these rabbits by 29th day of infection. Antibiotic therapy was effective in preventing the rise of gamma globulins and of blocking antibody through the 59th day.

1. Kuhns, W. J., *Am. J. Med.*, 1956, v20, 251.

2. Fitch, C. P., Donham, C. R., Bishop, L. M., Boyd, W. L., *Tech. Bull., Univ. Minn., Agri. Exp. Sta.*, 1930, v73, 1.

3. Wiener, A. S., *Modern Medical Monographs*, 1954, No. 9.

4. Feinberg, R., *J. Immunol.*, 1958, v81, 14.

5. Brahic, J., Tamalet, S., Madet, P., Girault, A., *C. rend. Soc. biol.*, 1955, v149, 373.

6. Cruickshank, J. C., *J. Hyg.*, 1956, v54, 562.

7. Conde, E., Pomales-Lebron, A., Cancio, M., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 640.

8. Royer, M., Molinelli, E. A., Noir, B., *Rev. Internat. D'Hepatol.*, 1957, v7, 599.

9. Ivanovskii, I. G., *Antibiotiki, Moskva*, 1957, v2, No. 4, 16.

Received March 13, 1959. P.S.E.B.M., 1959, v101.

A Single-Gene Antagonism Between Mother and Fetus in the Mouse.* (24966)

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Genetic antagonism between mother and fetus has not hitherto been demonstrated in the mouse(2,3). The purpose of the present paper is to demonstrate such a condition. This however differs from the classic types of incompatibility with Rh-antigens in man and erythrocytic antigens in livestock(1), since erythroblastosis fetalis and hemolytic icterus of the newborn has not appeared. A new sort of antagonism is therefore involved. Evidence for fetal antagonism appeared during genetic analysis of a new mutant type named "hair-loss" (Fig. 1). This is a simple recessive Mendelian character which originated as spontaneous mutation in this laboratory. It is characterized by progressive degeneration of hair follicles over entire body, loss of hair becoming obvious after age of about 5 weeks (Fig. 2). There is some resemblance to previously known mutant type, "hairless," gene *hr* in linkage Group III, but the new type, "hair-loss," proves to be in linkage Group VI, and has therefore been given a distinctive gene symbol, *hl*.

Methods. Homozygous hair-loss type has been maintained as a moderately inbred stock. For the present study, outcrosses have also been made with various other genetic types. Linkage-test matings for the VI group involved the marker genes *bt* ("belted"), *Ca* ("Caracul"), and *N* ("Naked"). Reciprocal matings served as controls. Standard mating has been one male with one female, usually not separated for littering. The young were weaned in a different box at about 4 weeks of age. In many cases, litters were recorded

* Journal Paper No. J-3559 of Iowa Agric. and Home Economics Exp. Station, Ames. This work received assistance from Contract No. AT(11-1) 107 from U. S. Atomic Energy Comm.