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Altered Immune Responsiveness Associated with *Encephalitozoon cuniculi* Infection in Rabbits

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The variation in immune response of two unrelated colonies of laboratory rabbits to high doses of heat-killed *Brucella abortus* strain 19 was investigated. One was a mixed-breed, multicolored colony in which a high prevalence of encephalitozoonosis had been recorded, whereas the other rabbits were derived from a colony of Dutch-marked specific-pathogen-free rabbits. Although considerable variation in the immune response between individual rabbits was noticed at all bleeds, rabbits infected with *Encephalitozoon cuniculi* showed, on comparison with uninfected rabbits from either colony, a depressed immunoglobulin G response from week 5 of the antigen injection schedule and, from week 8, an elevated immunoglobulin M response.

Seasonal and genetic variations in the immune responses of laboratory animals to injected antigens are reported frequently. In addition to the immune response genes (13), many external factors, especially nutrition, have been investigated as possible causes of these variations (3, 9). Furthermore, a general depression of the immune response has been shown to be associated with toxoplasmosis (10), malaria (8), trypanosomiasis (7), and a number of viral infections (18).

The present paper describes an altered immune responsiveness in rabbits naturally infected with *Encephalitozoon cuniculi*, an intracellular protozoan parasite (11). This organism can cause a disease of high prevalence in laboratory animals (4, 19) and is also pathogenic to many wild and domestic animals (19) and man (2). In rabbits, the disease is generally of a chronic, subclinical nature, although sporadic clinical outbreaks have been reported. Problems of recognition of encephalitozoonosis in living animals have been overcome recently by development of a serum immunofluorescence diagnostic test (4). Evaluation of this test (J. C. Cox and H. A. Gallichio, Res. Vet. Sci., in press) has shown a good correlation between presence of specific antibody and infection with *E. cuniculi*. In this paper the immune responses of rabbits with naturally acquired encephalitozoonosis have been compared with those of uninfected rabbits and found to have a depressed immunoglobulin G (IgG) response and an elevated IgM response in studies with *Brucella abortus* as immunogen.

MATERIALS AND METHODS

Rabbits. Healthy, 12-week-old rabbits for immune response studies were taken randomly from two unrelated colonies. Thirty rabbits were from a mixed-breed, multicolored colony, developed and maintained at the Commonwealth Serum Laboratories (CSL) over several decades. Encephalitozoonosis had been prevalent in this colony for a number of years (5). Ten Dutch-marked rabbits were derived from a nucleus of specific-pathogen-free (SPF) rabbits obtained in 1973 from the Central Institute for Laboratory Animals, Hanover, Germany. They were raised and maintained in isolation at CSL, and results of regular serum testing indicated that they continued to be free of *E. cuniculi*.

Serological diagnosis of *E. cuniculi* infection. All serum samples from all rabbits were tested by indirect immunofluorescence for the presence of antibodies to *E. cuniculi* at doubling dilutions from 1:10. The antigen used was spores of *E. cuniculi* liberated from infected cultures of fetal human fibroblasts. Detailed testing procedures have been reported elsewhere (4).

Immunization procedure. All rabbits were injected with heat-killed *B. abortus* strain 19 organisms (*Brucella abortus* bacterial suspension, CSL). Organisms were enumerated by use of Wellcome opacity tubes. Graded doses of from 2×10^9 to 8×10^9 organisms in 0.5 ml of saline were given intravenously on days 0, 3, 7, and 10, and then doses of 12×10^9 organisms were given at weeks 2, 4, 7, and 10. Rabbits were bled (1 to 2 ml) from an ear vein at weeks 1.5, 3, 5, 8, and 11.

***B. abortus* serum agglutination tests.** Saline and mercaptoethanol tube agglutination tests were performed essentially according to standard procedures (1). Individual titers were estimated from doubling dilution titrations and then determined with an accuracy of ±10% by retitration within this range. Anti-*B. abortus* IgG agglutination titers were taken as the agglutination titer in the presence of 2-mercaptoethanol, and anti-*B. abortus* IgM agglutination titers were calculated by subtraction of the titer of these mercaptoethanol-resistant antibodies from the saline agglutination titer for that serum. All titers are expressed in international units (IU) per

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milliliter.

Statistical treatment of results. Geometric means and *t*-tests were used in the analysis and presentation of IgG agglutination titers (Table 1). The IgM agglutination titers, obtained as described above, departed from a normal distribution to such an extent that similar treatment was not justified. The results (Table 2) are instead presented as median titer and range, and significance was determined by use of the Wilcoxon rank test (22).

RESULTS

The immune responses of 40 rabbits were studied, 30 from the CSL colony and 10 SPF-derived rabbits. Eleven rabbits from the CSL colony had high immunofluorescence titers to *E. cuniculi* at the start of this study, and a further six rabbits from this colony developed antibody titers to *E. cuniculi* within 3 weeks of initiation of immunization. Studies in progress suggest that the former group may have been infected since birth, whereas the latter group probably became infected 3 to 6 weeks prior to serum antibodies to *E. cuniculi* first being detected. These 17 rabbits all had high immunofluorescence titers to *E. cuniculi* at the termination of this experiment (range, 200 to 4,000), implying persistence of infection. The other 23 rabbits remained seronegative throughout the course of the study.

The results for the IgG and IgM responses to *B. abortus* of these four groups of rabbits are shown in Tables 1 and 2, respectively. All rabbits showed a typical response to *B. abortus* in the first 5 weeks, a high IgM and negligible IgG response by 1.5 weeks, and then a gradual loss of IgM antibodies as the secondary IgG response developed. In uninfected rabbits, this IgG response reached a plateau, which did not increase with further antigen stimulation. However, from week 5, both infected groups of rabbits showed a depressed IgG response to *B. abortus* when compared with the uninfected CSL group, and from week 8 both infected groups had an elevated IgM response to *B. abortus*. No significant differences were observed between the seronegative rabbits from the CSL and SPF-derived colonies. In all cases, significance of depression or elevation of the immune response was calculated by comparison with the appropriate response of the seronegative rabbits from the CSL colony.

DISCUSSION

The variation in immune responses between individual rabbits reported here appears similar to that reported elsewhere after immunization of rabbits with clostridial antigens (6) and guinea pigs with *B. abortus* (3). In these experiments, the effect of two factors on this variation were examined, namely, the strain of the rabbits and the state of natural infection with *E. cuniculi*. Uninfected rabbits from either colony, when dosed with *B. abortus*, showed similar immune responses.

The depression of the specific IgG response to an injected immunogen associated with a concurrent infection with *E. cuniculi* is paralleled by observations in other parasitic diseases (7, 8, 10). A number of mechanisms have been suggested to account for such observations in trypanosomiasis, including depression of Bγ-cell function (16), depression of "helper" T-cell function (14), and macrophage dysfunction or anti-

TABLE 1. *Influence of E. cuniculi infection on rabbit IgG anti-B. abortus immune response*

Rabbit colony	*E. cuniculi* infection	No. of rabbits	Geometric mean titer (IU/ml) at:			
			3 weeks[a]	5 weeks	8 weeks	11 weeks
CSL	Chronic	11	280 161–487[b] NS[c]	200 137–292 $P < 0.025$[d]	140 74–266 $P < 0.10$	88 36–214 $P < 0.025$
CSL	Recent	6	350 243–504 NS	200 128–312 $P < 0.05$	130 52–325 $P < 0.10$	175 71–430 NS
CSL	Uninfected	13	300 231–390	350 269–455	310 180–533	310 178–539
SPF	Uninfected	10	180 119–272 NS	540 325–896 NS	540 370–788 NS	375 188–746 NS

[a] Weeks from start of immunization.
[b] 95% confidence limits.
[c] NS, Not significant.
[d] Significance determined by comparison with the uninfected CSL group.

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TABLE 2. *Influence of E. cuniculi infection on rabbit IgM anti-B. abortus immune response*

Rabbit colony	*E. cuniculi* infection	No. of rabbits	Median titer (IU/ml) at:				
			1.5 weeks[a]	3 weeks	5 weeks	8 weeks	11 weeks
CSL	Chronic	11	4,000 (2,400–5,200)[b] $P < 0.01$[c]	1,300 (700–3,000) NS[d]	400 (0–1,400) NS	600 (0–1,900) $P < 0.05$	600 (40–3,600) $P < 0.05$
CSL	Recent	6	2,650 (1,800–4,600) NS	1,200 (600–2,900) NS	150 (0–500) NS	300 (140–800) $P < 0.02$	530 (260–840) $P < 0.01$
CSL	Uninfected	13	2,700 (2,000–4,200)	1,100 (500–2,800)	300 (100–600)	120 (0–360)	140 (0–360)
SPF	Uninfected	10	3,400 (1,100–6,100) NS	1,400 (600–2,900) NS	290 (60–520) NS	90 (0–380) NS	60 (0–420) NS

[a] Weeks from start of immunization.
[b] Numbers in parentheses give the range.
[c] Significance determined by comparison with the uninfected CSL group.
[d] NS, Not significant.

genic competition (20). The elevation of the specific IgM response to *B. abortus* associated with *E. cuniculi* infection observed in this study has some precedent in studies on other parasitic diseases where an increase in largely nonspecific IgM has been noted (17). However, Murray et al. (16) reported an absence of anti-sheep erythrocyte IgM in *Trypanosoma brucei*-infected mice 5 days after immunization, and Longstaffe et al. (12), in similar experiments, found a decrease in IgM plaque-forming cells in *T. brucei*-infected, immunized mice.

The effect of *E. cuniculi* infection upon the immune response of rabbits to injected *B. abortus* organisms may involve both antigenic competition and macrophage dysfunction. The constant release of *E. cuniculi* from various foci of infection throughout the body would compete with injected *B. abortus* for "uncommitted" macrophages and cause a shortage of macrophages available to process *B. abortus* antigen. Also, like *Toxoplasma gondii* (10), *E. cuniculi* is capable of growth within macrophages (19, 21) and thus could cause a defect in the ability of the macrophages to process and/or present injected antigens to lymphocytes. Both factors would inhibit a T-cell-mediated switch from IgM to IgG synthesis and lead to the depressed IgG and elevated IgM responses observed here.

A reduction in macrophage function or depletion of macrophages would also result in a longer survival of *B. abortus* in the circulation. Thus, through increased persistence in lymphoid organs, the antigens may lead to induction in B-cell precursors of low-affinity, IgM antibody-secreting cells but tolerance in many of body-secreting cells. This could stimulate synthesis of IgM to new determinants on the surface of *B. abortus* and further depress IgG synthesis. The effect would simply reflect different fates of B-cells with different antigen-binding capacities. T-cells could also be the targets for inhibition by persistent antigens (15).

Most of the results presented here do not show as severe an immune depression as reported for other parasitic infections. This may mean that *E. cuniculi* causes a lesser immune depression than infection with other more pathogenic intracellular protozoa or may be because the seropositive animals in the above experiments were naturally infected, probably since birth, rather than given an acute artificial infection, as has generally been the case in studies on other parasites.

The results in this paper strongly suggest that infection of laboratory animal colonies with *E. cuniculi* can increase the variability of experimental results. Eradication of infection, which should be possible by means of serological screening of colonies with elimination of infected animals, would therefore seem warranted.

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