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Protection of mice against haemoprotozoan *Babesia microti* with *Brucella abortus* strain 19

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SUMMARY

When *Brucella abortus* strain 19 is given intraperitoneally to mice it protects them against subsequent infection with large doses of *Babesia microti*. The protection obtained was more effective when *B. abortus* was given intraperitoneally than when it was injected subcutaneously. This non-specific protection seems to be best explained by the stimulation of macrophages so as to release a mediator which limits the intracellular replication of the parasites.

INTRODUCTION

Mice infected with *Brucella* spp. are subsequently resistant to infections with phylogenetically unrelated organisms. This type of non-specific immunity has been noted between many pairs of organisms. Thus mice infected with *Besnoitia jellisoni* are resistant to *Listeria monocytogenes* (Ruskin, McIntosh & Remington, 1969), mice infected with *Mycobacterium tuberculosis* are resistant to *Salmonella enteritidis* (Howard *et al.*, 1959), those infected with *S. typhimurium* are resistant to *L. monocytogenes* (Zinkernagel, 1976) and those infected with *M. bovis* (strain BCG) are resistant to *Babesia microti* (Clark, Allison & Cox, 1976). Non-specific immunity has also been produced by killed organisms (e.g. *Corynebacterium parvum* against *Babesia* and *Plasmodium* spp.; Clark, Cox & Allison, 1977) or by injections of lipopolysaccharide (Berger *et al.*, 1969) and various other microbial extracts (Dubos & Schaedler, 1957).

The results now presented show that mice infected with an attenuated vaccine strain of *Brucella abortus* (strain 19) are strongly protected against infection with *Babesia microti*.

MATERIALS AND METHODS

Animals. Female 5–6-week-old CBA/Ca strain mice were used in most experiments, although age and sex did not appear to influence susceptibility to *B. microti*. Mice were bred in SPF conditions at the Clinical Research Centre, Harrow, from Carshalton stock. During the course of the experiments stock mice were splenectomized and shown to be free of *Eperythrozoon* spp.

Protozoa. Parasitized red cells containing *B. microti* (Kings' strain) were stored as a 50:50 mixture with 4 M dimethylsulphoxide in liquid nitrogen in volumes of 200 μl. When required, these aliquots were thawed in a 37°C waterbath and immediately injected intraperitoneally into a mouse. While its parasitaemia was rising this mouse was used as a donor to experimental mice.

Mouse parasitaemias were monitored every second day by Giemsa staining of thin blood smears and were expressed as the percentage of red cells containing one or more parasites.

Brucella abortus vaccine. Freeze-dried strain 19 (S19) *B. abortus* (Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey) was reconstituted with phosphate-buffered saline just before use to $2{\cdot}3 \times 10^9$

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viable units/ml. Unless otherwise stated, 250 μl of this suspension was given to mice intraperitoneally. Vaccination and all subsequent manipulations with *B. abortus*-infected mice were carried out in an exhaust-ventilated cabinet with glove portholes.

Indirect fluorescent antibody (IFA) titrations. The method of Voller & O'Neill (1971) was used, the only modification being a 0·1% Evan's blue counterstain. The anti-globulin conjugate (rabbit anti-mouse IgG) was prepared as described by Cox, Crandall & Turner (1969).

RESULTS

Route of injection of B. abortus

Nine mice were given $5{\cdot}7 \times 10^8$ viable units (vu) of *B. abortus* S19, four mice by the intraperitoneal and five mice by the subcutaneous route. 14 days later these two groups and five untreated mice were infected with 10^8 *B. microti*. Those given bacteria i.p. were more resistant to *B. microti* than those injected s.c. (Fig. 1).

Interval between B. abortus and B. microti

B. abortus ($5{\cdot}7 \times 10^8$ vu) was given to four mice 42 days, and to five mice 13 days, before injection with

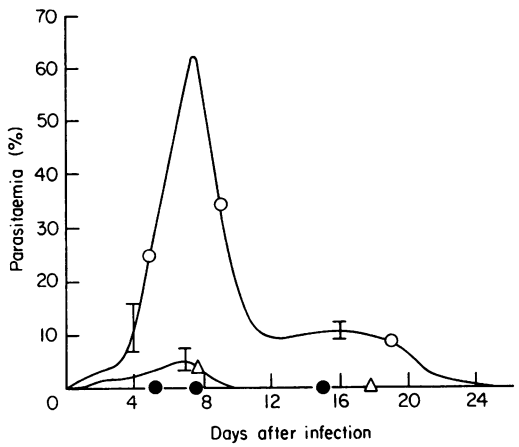

FIG. 1. 10^8 *B. microti* given 14 days after pretreatment with $5{\cdot}7 \times 10^8$ vu of *B. abortus* by different routes. (△) s.c. (●) i.p., (○) controls with no *B. abortus*. Graphs show geometric mean parasitaemias, and vertical bars indicate the standard error of a mean.

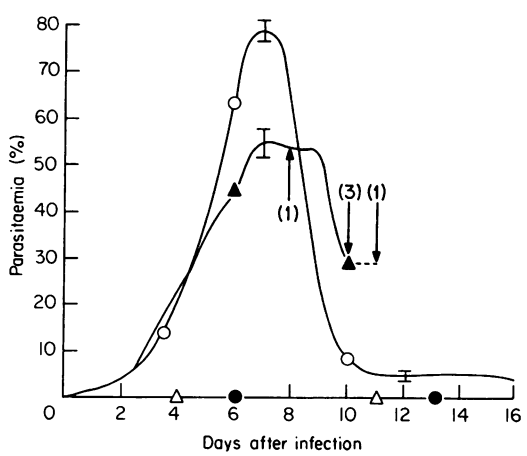

FIG. 2. Various time intervals between i.p. *B. abortus* ($5{\cdot}7 \times 10^8$ vu) and $1{\cdot}2 \times 10^8$ *B. microti*. *B. abortus* given (△) 42 days before *B. microti*, (●) 13 days before *B. microti*, (▲) 20 hr after *B. microti* (numbers in brackets show how many mice were dead on the days indicated by the arrows), (○) controls with no *B. abortus*.

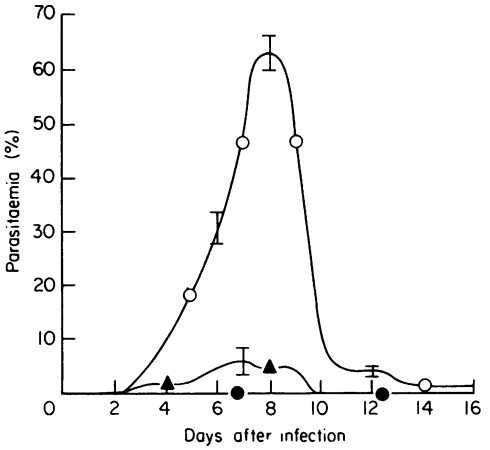

FIG. 3. $0{\cdot}5\times10^8$ *B. microti* given 20 days after different doses of i.p. *B. abortus*. (●) $5{\cdot}7\times10^8$ vu *B. abortus*, (▲) $7{\cdot}8\times10^5$ vu *B. abortus*, (○) controls with no *B. abortus*.

TABLE 1. Anti-*B. microti* antibody levels

Time serum taken	Titre
29 days after *B. abortus* alone	< 1:10
9 days after *B. microti* (*B. abortus* 20 days before *B. microti*)	1:40
15 days after *B. microti* (*B. abortus* 20 days before *B. microti*)	1:5120
22 days after *B. microti* (*B. abortus* 14 days before *B. microti*)	1:10240
9 days after *B. microti* alone	1:640
15 days after *B. microti* alone	> 1:20480
22 days after *B. microti* alone	> 1:20480

$1{\cdot}2\times10^8$ *B. microti*. This number of parasitized red cells was also given to five control mice. Another group of five mice were given $5{\cdot}7\times10^8$ vu of *B. abortus* 20 hr after infection with this dose of *B. microti*. Protection was total when *B. abortus* was given 13 or 42 days before *B. microti*. While *B. abortus* given 20 hr after *B. microti* provided slight protection, all these mice died during the course of the infection (Fig. 2).

Dose of B. abortus

Two groups of four mice were given $5{\cdot}7\times10^8$ and $7{\cdot}8\times10^5$ vu of *B. abortus* S19 respectively. 20 days later these two groups and four untreated mice were infected with $0{\cdot}5\times10^8$ *B. microti*. As previously, the higher dose gave absolute immunity, and protection was appreciable also with the lower dose (Fig. 3).

Duration of protection

Four mice protected from an initial challenge with 10^8 *B. microti* by $5{\cdot}7\times10^8$ vu of *B. abortus* S19 given 14 days earlier were subsequently rechallenged with $1{\cdot}2\times10^8$ *B. microti* 71 days later. Protection was again almost absolute, with a peak geometric mean parasitaemia of 1·6%. This was significantly different from that of the control group.

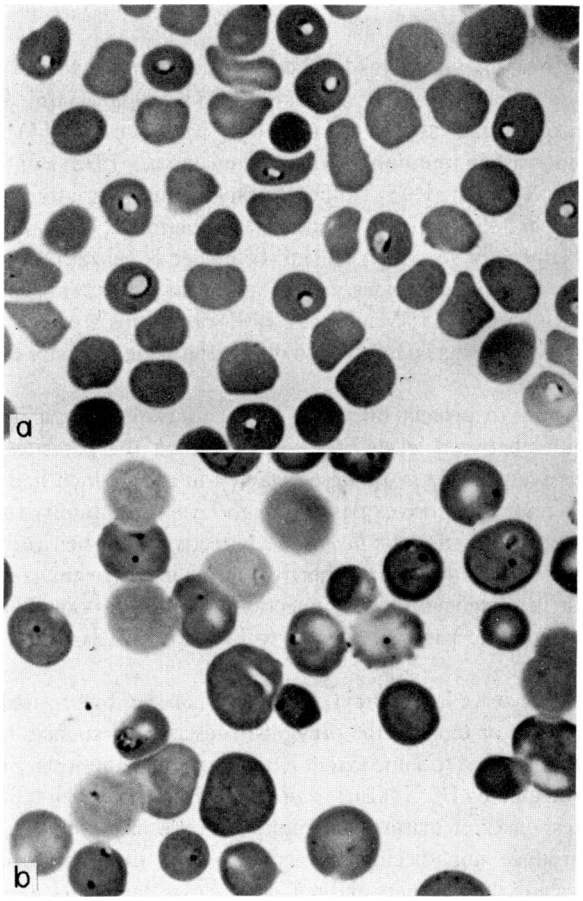

FIG. 4. (a) Normal *Babesia microti*. (b) Degenerate *Babesia microti* inside red cells of mice protected by pretreatment with Strain 19 *B. abortus*. (Magnification × 700 for both).

Anti-B. microti antibody levels (IFA titres)

Antibodies to *B. microti* antigen were measured in serum collected at various times after *B. microti* had been given to mice which had earlier received $5{\cdot}7 \times 10^8$ vu of *Br. abortus*. As shown in Table 1, titres were lower than those of the controls given *B. microti* alone, yet controls had much higher parasitaemias. Sera from four mice were pooled for each reading.

Presence of intra-erythrocytic pyknotic forms

As in mice pretreated with BCG (Clark *et al.*, 1976) or *Corynebacterium parvum* (Clark, Cox & Allison 1977), parasites with a pyknotic appearance on light microscopy and confirmed by electron microscopy to be dead inside red cells (Clark *et al.*, 1975) were associated with both recovery of control groups and protection by prior infection with *B. abortus* S19 (Fig. 4).

DISCUSSION

The results have shown that prior infection of mice with *B. abortus* S19 apparently confers complete protection against subsequent *B. microti* infection, provided that the bacteria are given by the procedure outlined. This involved giving $5{\cdot}7 \times 10^8$ *B. abortus* by the i.p. route between 13 and 42 days before

injecting the piroplasm. Although there is no doubt about the efficiency of the protection obtained, there is some uncertainty as to its explanation.

Injection of mice with *Brucella* organisms has been shown by many workers to elicit a variety of host responses. These include splenomegaly (Feldman & Olson, 1935; Gafni, Olitzki & Ikowicz, 1960; Toujas *et al.*, 1972), macrophage 'activation' (Khoo & Mackaness, 1964), increase in phagocytosis (Passos *et al.*, 1963), an increase in immunoglobulins (Toujas *et al.*, 1972) and the production of lymphokines (Sandok, Hinsdill & Albrecht, 1975). Animals respond similarly after injections of other organisms such as BCG (Old *et al.*, 1961; Miller, Mackaness & Lagrange, 1973; Salvin, Nishio & Shonnard, 1974) and *C. parvum* (Halpern *et al.*, 1964; Neveu, Bronellec & Biozzi, 1964; Cerutti, 1974).

For the demonstration of these responses it does not seem to matter whether killed (Toujas *et al.*, 1972) or live (Feldman & Olsen, 1935; Mackaness, 1964) organisms are used. However, it does appear that a larger dose of killed organisms is required to obtain the same response as that obtained with living organisms (Sulitzeanu, 1965).

B. abortus did not appear to protect by acting as an adjuvant, increasing antibody formation: no correlation could be found between levels of antibody against the parasites and protection. Also no cross-reacting antibodies against *B. microti* were detected in mice which had received only *B. abortus*. Direct antibody action cannot therefore explain the high level of immunity to this piroplasm produced by *B. abortus*. The timing of the effect of *B. abortus* (effectiveness when administered long before the parasite and lack of effect when administered shortly after it) is unlike any conventional adjuvant effect. Furthermore, an increase in phagocytosis after injection of *B. abortus* cannot account for the protection against *B. microti* since pyknotic parasites, shown to be dead by Clark *et al.* (1975), are seen inside red cells in the blood stream.

However, the protection that we have obtained against *B. microti* by pre-treating mice with *B. abortus* S19 is probably related to one or more of the previously described responses to this bacterium. Perhaps all these host reactions are involved to some extent in resistance to babesiosis. Since congenitally thymus-deprived mice can be protected by BCG (Clark *et al.*, 1977) and *C. parvum* (Clark, Cox & Allison, 1977), it seems unlikely that responses of mature T lymphocytes are important. A common feature with all these organisms that produce non-specific protection against haemoprotozoa is that they stimulate macrophages, both increasing the numbers and performance of these cells, and result in intra-erythrocytic death of the parasites. We suggest that a factor released by macrophages stimulated *in vivo* by *B. abortus* limits the multiplication of *B. microti* in erythrocytes, perhaps by blocking the transport of nutrients essential for parasite growth across the red cell plasma membrane. Experiments to test this hypothesis are in progress in our laboratory.

In addition to being protected against *B. microti*, mice infected with *B. abortus* S19 have increased resistance to *M. tuberculosis* (Nyka, 1956), *L. monocytogenes* (Mackaness, 1964) and tumours (Hirnle, 1960; Toujas *et al.*, 1972) and vaccinia virus (Billiau *et al.*, 1970). The bacterium also protects guinea-pigs against *Coxiella burnetii* (Mika *et al.*, 1954). In no case, however, was immunity as strong as that which we have obtained with *B. microti*. Hence this combination of *B. abortus* S19 and *B. microti* appears to be a particularly useful model for further elucidation of the principles underlying this type of non-specific immunity, as well as being a possible basis for control of haemoprotozoa in other hosts.

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