GENETIC RESISTANCE TO BABESIOSIS

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SUMMARY

Mechanisms of resistance to babesiosis include protection by antibody, opsonization and phagocytosis of infected cells and possibly complement mediated lysis of infected cells. These immune mechanisms are controlled by elements in the major histocompatibility complex suggesting genetic resistance is a factor in modulating the disease caused by Babesia spp. The direct evidence that genetic resistance is important lies with the findings that Bos taurus and Bos indicus cattle differ in their response to B. bovis and perhaps B. bigemina. Since the disease can be controlled cheaply and efficiently by vaccination, genetic resistance has not been exploited as a control measure. The potential danger of babesiosis occurring when cattle are selected for resistance to ticks is apparent and therefore it is of some interest that resistance to B. bovis is weakly correlated to resistance to its vector Boophilus microplus.

INTRODUCTION

Smith and Kilborne (1893) reported Babesia to be the cause of Texas Fever in cattle in USA and devised means of controlling the disease by infecting cattle with parasitized blood. After the introduction of babesiosis into Australia with ticks (Boophilus microplus) in the early 1900's the disease was quickly brought under control using the same techniques (Pound, 1908). Since then the scientific literature on babesiosis has grown, with most interest centering on the immune responses of the host to the parasite. This has been reviewed recently by Zwart and Brocklesby (1979). A major advance has been in improving vaccination procedures as described by Callow (1977). Mahoney (1967) showed that protection against Babesia bovis could be induced by injecting crude extracts of the parasite. This has led to work on isolating and characterizing the protective antigens (Wright et al., 1985) which in turn should lead to production of sub-unit vaccines produced by genetic engineering. These developments have precluded other approaches to control of babesiosis and not much research has been done on genetic resistance to the disease.

IMMUNE MECHANISMS OF RESISTANCE TO BABESIOSIS

Antibody.

The presence of antibody following immune responses against babesiosis has been shown in several systems. Cox and Turner (1970) using immuno-fluorescent antibody tests found IgM and IgG antibodies in mice soon after infection with B. microti. Evidence of a shift from IgM to IgG synthesis was not observed. However, Mahoney (1972) made a preliminary study of the antibody classes concerned with the response of cattle to the hemagglutinating antigens of B. bovis, and found IgM the predominate antibody class at 3 weeks, whereas IgG predominated 6 months after infection.
The period after infection with Babesia in which antibodies are detectable depends on the sensitivity of the serological method used and perhaps the antigenic relationship between host and infecting parasites. Regardless of species of Babesia, host, or method of detection, the quantity of circulating antibody measured by titration of serum reaches an early peak and gradually declines even though parasites persist in fluctuating numbers in the blood, exposing the host to a continuous antigenic stimulus.

Evidence that antibodies are concerned with protection against Babesia was first provided by Hall (1960, 1963) who demonstrated passive transfer of immunity to B. bovis from cow to calf, presumably by antibodies in colostrum. Since then, a number of workers have demonstrated passive transfer of immunity in mice to B. rodhaini (Matson, 1964; Ludford, 1967; Phillips, 1969b) and in cattle to B. bovis (Mahoney, 1967a) with serum from infected animals. In both of these host parasite systems, protection was manifested by a delay in the onset of parasitemia if antisera was administered at the time of infection, and was enhanced when serum administration was delayed for several days until parasites were detected in the peripheral blood. With B. bovis, protective activity of antisera was increased by superinfection of donors. However, there was no cross-protection in passive transfer tests between two strains of B. bovis isolated from different localities in Australia, even though infection with one strain conferred active protection against the other.

Mahoney (1972) reported that B. bovis-infected erythrocytes did not lose infectivity for splenectomized calves after incubation with antisera that protected cattle in passive transfer tests. In addition, lysis of the infected erythrocytes with sheep anti-erythrocyte antisera and complement prior to their treatment with immune serum did not alter their infectivity. In another experiment described by Mahoney (1972), calves were immunized with normal erythrocytes from one donor calf and were later inoculated with B. bovis-infected blood from this donor. The presence of antibodies to the erythrocytes that contained the infecting Babesia did not alter the subsequent course of infection in these calves. Unlike the antibodies in mice and rats, antibodies in cattle against erythrocytes of the inoculum were not protective. However, he postulated there could be a difference between cattle and mice in the way parasitized erythrocytes are removed from the circulation. In some circumstances antibody-coated erythrocytes are merely sequestered and not destroyed (Mahoney, 1972) so if this happened to the infected erythrocytes in cattle, the Babesia would have been able to multiply.

Enhancement of infection with B. rodhaini after treatment of rats with immune serum was first observed by Ludford (1967). It was not a constant feature of his passive transfer experiments but similar findings were also observed by Roberts (1968) in the same host-parasite system. The latter author suggested that different types of antibody, antagonistic in action and produced in different proportions, might be involved.

Phagocytosis.

There has been a tendency to accept the description of cellular events in malaria (Taliaferro and Cannon, 1936) as generally applicable to babesiosis. Although the available descriptions provide some justification for this attitude more studies of phagocytosis in Babesia infections should have been undertaken. The most important function of phagocytosis might be the removal of infected erythrocytes from the circulation after the combination of antibodies with antigen on the erythrocyte surface, and through this mechanism the control of parasite multiplication.
Most descriptions of phagocytic activity in babesiosis have concerned B. canis infection in dogs (Neitz, 1938; Maegraith et al., 1957), but some observations have been made in mice with B. rodhaini (Paget et al., 1962) and in cattle with B. bovis (Karput, 1966; Rogers, 1971b). The cellular reactions described include mobilization of mesenchymal reserves with hyperplasia of reticulo-endothelial (RE) elements in the spleen, liver, and bone marrow. Phagocytosis of non-parasitized and parasitized erythrocytes by macrophages occurs in these organs and in the medulla of the lymph nodes. There is depletion of lymphocytes of germinal centres in the latter organs and in the spleen at this time. These general changes are probably a part of the early manifestation of natural immunity, an immediate response of the RE system to substances liberated by parasites. The phagocytosis of infected and non-infected erythrocytes may thus commence as a non-specific reaction accompanying a rise in the general level of activity of those elements normally responsible for the removal of damaged and aged erythrocytes. As infection progresses, there is intensification of phagocytic activity particularly in the spleen, liver, and bone marrow, and plasma cells appear in these organs (Rogers, 1971b). In the blood, lymphocytosis may occur (Karput, 1966; Dorner, 1967), and monocytes and neutrophils ingest free parasites as well as infected and non-infected erythrocytes (Neitz, 1938). Production of specific opsonic antibody for parasites may be expected to accompany such changes. Adherence of plasma antigens to normal erythrocytes of dogs and rats infected with Babesia was demonstrated by Sibinovic et al. (1969). They considered this suggested an immune mechanism for indiscriminate removal of erythrocytes in the above hosts during late stages of infection. The antibodies were lytic for antigen-sensitized cells in vitro, but they postulated opsonization might also occur in the infected animals.

The role of the spleen in immunity to Babesia has not been clearly defined. In recent years studies directly concerned with splenic function in B. rodhaini infection in rats (Todorovic et al., 1967; Phillips, 1969a) have found that important splenic activities were phagocytosis and rapid formation of antibody to blood-borne antigens, a property originally described by Taliaferro and Stauber (1969). Phagocytosis was mostly the province of the spleen in the early phase of infection with Babesia, but other organs, for example liver and bone marrow, performed this function equally well at a later stage. Another function of the spleen may be in removing infected erythrocytes by rheological properties of the infected cell.

Complement and Conglutinin.

The important role that complement plays in the immune response to Babesia has been shown in critical experiments with B. bovis in cattle (Mahoney et al., 1980; Goodger et al., 1981). Conglutinin, a natural opsonin present in large amounts in cattle serum, also plays a role in clearance of infected or damaged erythrocytes as does fibronectin in the sequestration of erythrocytes (Goodger et al., 1981).

Inheritance of Immune Mechanisms to Babesiosis.

Specific genetic factors that alter host susceptibility to babesiosis have not been determined in domestic animals. A recent review of the function and genetics of the major histocompatibility complex by Giles and Capra (1985) shows that antibody and complement production, and macrophage function are under the control of genes located in or near this complex. Although most of the evidence for this pertains to murine and human systems it is highly likely
that similar control will exist in other species. Susceptibility of cattle to babesiosis varies in different genetic types. In Australia, Bos indicus cattle are more resistant than Bos taurus cattle to B. bovis infection (Daly and Hall, 1955), and a low incidence of babesiasis has been reported among these cattle in the field (Francis and Little, 1964; Johnston, 1967). In Africa, Lohr (1973) reported the high innate resistance of Sahiwal cattle (Bos indicus) to B. bigemina. The study of Johnston (1967) in parallel surveys of comparable groups of Bos indicus x Bos taurus and Bos taurus cattle demonstrated a significant difference in incidence of B. bovis parasitemia. However, experience in Australia and also elsewhere (Dumag et al., 1962; Schiffo and Lombardero, 1964; Davidson, 1969; Rajamanickam, 1970; Ranatunga and Wanduragala, 1972) has shown that babesiosis can be a serious disease in previously unexposed (Bos indicus x Bos taurus) cattle, and in such populations of predominantly cross-bred animals the overall effect of breed factors in resistance requires further evaluation.

Indirect evidence for a genetic basis of resistance rests with the variability of the immune response to babesiosis. For example the onset of detectable parasitemia following primary infection with 10⁶ B. bovis can vary between animals by up to 48 hours. The basis for this is unknown but it could be linked to receptors on bovine erythrocytes determined by their blood type.

EXPERIMENTAL EVIDENCE FOR GENETIC RESISTANCE TO BABESIOSIS IN CATTLE

1. Primary infections. Following the observations of Daly and Hall (1955) no further work was reported on differences in response to experimental infections with B. bovis until the paper by Johnston and Sinclair (1980). They reported an experiment on 36 cattle which compared the response of Hereford, Droughtmaster (½ Brahman x Shorthorn cattle) and Brahmans to primary infection with B. bovis. Half of each group was splenectomized in order to investigate the underlying mechanisms of resistance. As well, this procedure enabled a critical look at the immune response without having a large number of challenge doses in the experiment which would have been prohibitive in terms of the numbers of cattle required. The statistical analysis used was a canonical variate analysis which gave a better understanding of the four variables which were measured to estimate resistance. This was an interesting experiment because if the splenectomised cattle had not been included then no significant difference between the breeds was apparent. However, on complete analysis the Brahmans were significantly more resistant than the other two breeds. This is an important result as it indicates that cross-bred B. indicus x B. taurus cattle are as susceptible to primary infection as B. taurus cattle.

2. Latent infections. No experimental data is available comparing the duration of latent infections in Bos indicus cattle to Bos taurus cattle. Johnston et al. (1978) reported a comparison between Hereford and Droughtmaster cattle. Ten Droughtmaster and nine Hereford cattle were born in an enzootic babesiosis area and became naturally infected with Babesia bovis and B. bigemina during a 3 year period. They were then kept free of cattle ticks (Boophilus microplus) for the remainder of the experiment. Annually for the next 3 years their individual infection status with Babesia was determined by sub-inoculation of blood into splenectomized calves. At the end of this period the functional immunity of all cattle was challenged by blood inoculation of heterologous strains of B. bovis and B. bigemina. Infection with B. bovis persisted in all Herefords for 2 years and in seven for 3 years after they had been freed of B. microplus. The number of Droughtmasters with detectable B.
bovis infection progressively declined, and at the end of 3 years only two of 10 were still infected. No Herefords were shown to be infected with B. bigemina following 1 year's freedom from B. microplus but latent B. bigemina infection of at least 2 years' duration was demonstrated in one of the Droughtmasters. A marked degree of resistance was apparent in all cattle when they were challenged with an heterologous strain of B. bovis. There were no differences between the response to challenge of the Herefords and Droughtmasters nor between the reactions of cattle which had apparently naturally sterilized B. bovis infection and those which were still infected. The heterologous strain of B. bigemina produced parasitemia in the majority of animals but only minimal fever and anemia resulted with no significant differences between the breeds.

CORRELATIONS BETWEEN RESISTANCE TO PRIMARY INFECTION WITH B. BOVIS AND ACQUIRED RESISTANCE TO THE CATTLE TICK (BOOPHILUS MICROPLUS)

Twenty-seven Droughtmaster cattle were challenged with B. bovis as described by Johnston (1978). They had been born and raised in a tick-free area at Magnetic Island in northern Australia and varied in age from adult cows to 6 month-old calves. Following this challenge the animals were transported to “Lansdown”, a CSIRO field station and allowed to become naturally infested with ticks. After exposure to ticks for 6 months, standard ticks were counted three times as described by Wharton and Utech (1970). The correlations between the parameters measured following Babesia challenge and tick counts are shown in Table 1. Low parasitemia, low rectal temperature and high antibody titre were weakly correlated with tick resistance as were high levels of anemia. The last finding is difficult to explain but the other parameters indicate that selection for tick resistance may be positively linked to selection for tick fever resistance.

Table 1. Correlations between response to primary infection with Babesia bovis and resistance to Boophilus microplus.

<table>
<thead>
<tr>
<th>Animal Age</th>
<th>Number of Animals</th>
<th>Parasitemia</th>
<th>Rectal Temperature</th>
<th>Packed Cell Volume</th>
<th>Antibody Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>8</td>
<td>0.241</td>
<td>0.230</td>
<td>-0.411</td>
<td>-0.876</td>
</tr>
<tr>
<td>6 months</td>
<td>6</td>
<td>0.043</td>
<td>-0.062</td>
<td>-0.101</td>
<td>-0.337</td>
</tr>
<tr>
<td>1 year</td>
<td>5</td>
<td>0.887</td>
<td>0.684</td>
<td>0.058</td>
<td>-0.497</td>
</tr>
<tr>
<td>2 years</td>
<td>6</td>
<td>0.152</td>
<td>0.732</td>
<td>0.112</td>
<td>0.278</td>
</tr>
<tr>
<td>Overall</td>
<td>27</td>
<td>0.297</td>
<td>0.257</td>
<td>-0.144</td>
<td>-0.315</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSIONS

There is good experimental evidence that in the absence of repeated infection Bos indicus × Bos taurus cattle sterilized their infections with B. bovis quicker than Bos taurus cattle. This is probably not a disadvantage as these cattle were able to withstand a heterologous challenge. On the other hand Bos indicus cattle are more resistant to primary challenge than Bos taurus or Bos taurus × Bos indicus crosses. Correlations between resistance to primary infection with B. bovis and resistance to ticks (Boophilus microplus)
suggest that selection for tick resistance will also enhance tick fever resistance. Because cheap and effective vaccines against babesiosis are available it is most unlikely that selection for this trait will be used to improve livestock production. However, selection for tick resistance may be positively linked to resistance to tick-fever thus making the tick resistance trait an even more attractive aid to productivity.

REFERENCES


