SERUM OPSONINS AND THE PASSIVE TRANSFER OF PROTECTION IN \textit{BABESIA RODHAINI} INFECTIONS OF RATS

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Abstract—ROGERS R. J. 1974. Serum opsonins and the passive transfer of protection in \textit{Babesia rodhaini} infections of rats. International Journal for Parasitology 4: 197–201. An investigation into the protective activity of serum from rats immune to \textit{B. rodhaini} and the role played by opsonins in that activity was undertaken. One, three and six infections with \textit{B. rodhaini} resulted in corresponding increases in the titre of specific protective antibody demonstrable by the administration of immune serum to rats. Drug control of infection resulted in a lower level of protective activity than that which developed when rats controlled infection unaided. Protective activity following recovery from a single drug controlled infection was undiminished 20 weeks after infection.

Serum opsonins were detected in an \textit{in vitro} culture system of normal rat peritoneal macrophages and these antibodies were specific for parasitized erythrocytes. It is suggested that opsonins were largely responsible for the protective effect demonstrated by assay in rats but that their importance, relative to other antibodies with a possible protective function, in the development of acquired immunity remains to be determined.

INDEX KEY WORDS: \textit{Babesia rodhaini}; opsonins; passive transfer; protection; rats.

INTRODUCTION

Some protection against \textit{Babesia rodhaini} (Phillips, 1969; Roberts \textit{et al.}, 1972) and against \textit{B. argentina} (Mahoney, 1967) has been conferred by the passive transfer of immune serum. Mahoney (1967) found that hyperimmunization increased protective activity in bovine babesiosis. However, neither the degree to which the protective effect can be enhanced by hyperimmunization of the donor nor the role of anti-erythrocyte antibody in rodent babesiosis have been fully elucidated. Similarly the mechanisms by which this effect is mediated have not been determined.

In order to assess the possible significance of opsonizing antibodies in the protective activity of serum from rats immune to \textit{B. rodhaini}, an \textit{in vitro} macrophage culture system was set up in which the activity of serum opsonins could be studied. Antisera produced by hyperimmunizing rats both with \textit{Babesia} infected erythrocytes and normal erythrocytes were tested in this system and their protective activity was determined by passive transfer assay methods in rats.

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Production of antisera

Inoculation schedules used to produce antisera were as follows:

(i) Anti-Babesia infected erythrocyte serum (anti-Babesia serum). Thirty rats were infected with the reference population of *B. rodhaini*. Infections were treated on day 4 with amicaralide isethionate ('Diampron', May & Baker) at a dose rate of 10 mg/kg. Eighteen of these rats received no further treatment and groups of six were bled at intervals of 5, 11 and 20 weeks after the primary infection. Six of the remaining rats received two further infections at 5 and 6 weeks following primary infection and were bled after a further 5 weeks. The final six rats were also reinoculated at 5 and 6 weeks and thereafter at 3-weekly intervals until they had received a total of six infections. They were bled 5 weeks after the final inoculation at 20 weeks after the primary infection.

Serum collected from six rats which had recovered from infection unaided 5 weeks previously was also pooled.

(ii) Control sera. Three groups of four rats each were inoculated with normal erythrocytes and one group was bled 5 weeks later. The remainder were subjected to the same inoculation and bleeding schedule as that described in the first paragraph of section (i) above. These rats received the same number of erythrocytes as did the *Babesia* infected rats.

Twelve rats were used as donors of normal serum and they were bled, in groups of four, 5, 11 and 20 weeks after primary inoculation of the other rats.

Four rats were injected with 'Diampron' at 10 mg/kg at the same time as the *Babesia* infected rats and were bled 5 weeks later.

Assay of protective activity of antisera in rats

The methods used were based on those of Phillips (1969). Erythrocytes containing \(5 \times 10^6\) parasites in 0·5 ml PBS were added to tubes containing 0·5 ml, or a 1:10 dilution in PBS, of the serum under test. The tubes were kept at room temperature for 90 min after which the contents of each individual tube were inoculated into a separate rat. Four recipient rats were used for each serum, or serum dilution under test except in the assay of six time hyperimmune serum at a final dilution of 1:2 when five were employed.

Serum collected from rats inoculated with normal erythrocytes and from normal untreated rats of the same weight as the donors of anti-*Babesia* serum were included in each assay. The protective activity of antisera was assessed as the mean time to 2 per cent parasitaemia in excess of that of the recipients of the appropriate anti-normal erythrocyte control serum. Data for the various groups in each assay were analysed by the t-test (Snedecor, 1956).

Opsonization test

The technique used was based on that described by Brown et al. (1970) Normal rats were killed by dislocation of the neck and macrophages were flushed from the peritoneal cavity with tissue culture medium '199' (British Drug Houses) containing 6 i.u. of heparin/ml. Approximately 10⁶ cells in 1·0 ml of medium were added to Leighton tubes containing coverslips. A quantity of 0·2 ml of the serum under test, or a dilution of the serum in foetal calf serum, was added to each tube. Serum was tested undiluted and then in doubling dilutions commencing at 1:10. The tubes were incubated at 37°C for 2 hr and then 50 \(\times 10^6\) parasites derived from blood with at least a 70 per cent parasitaemia, or 50 \(\times 10^6\) normal erythrocytes, in 1·0 ml of '199' were added and the tubes reincubated for a further 2 h. Coverslips were removed, rinsed, fixed and stained with Giemsa. The phagocytic index was estimated as the mean number of erythrocytes phagocytosed per peritoneal cell and was based on a count of 200 such cells. Normal serum and foetal calf serum controls were included with all tests and a serum was classified 'positive' if the phagocytic index exceeded 0·2.

RESULTS

Assay of protective activity of antisera in rats

None of the control sera had any effect on the mean time to 2 per cent parasitaemia of recipient rats and the results for all groups are shown in Table 1. Serum collected from rats 5 weeks after drug controlled primary infection had a significant protective effect \((P < 0·01)\) and hyperimmunization with the reference strain resulted in significant corresponding increases in protective activity demonstrated in antisera at final dilutions of 1:2 \((P < 0·05)\) and 1:20 \((P < 0·001)\). Protective activity was demonstrated at approximately the same level at 20 as at 5 weeks after drug controlled primary infection without hyperimmunization but serum taken 5 weeks after infection from rats which recovered unaided had significantly \((P < 0·01)\) greater activity.

Prepatent periods in rats receiving anti- *Babesia* serum were prolonged. Once infections became patent multiplication proceeded at a slightly slower rate than in control rats and infections were usually controlled at significantly lower peak parasitaemias and there were fewer deaths (Table 2). This effect was most marked in those rats receiving hyperimmune sera. Two out of five rats given hyperimmune anti-*Babesia* serum diluted 1:2 in Assay 3 did not develop patent parasitaemia and resisted challenge on day 28 with 500 \(\times 10^6\) parasites. Infection was not established in three rats, from 2 groups in Assay 3, because of faulty injection technique. They proved to be fully susceptible to challenge infection.

Testing of sera for opsonic antibodies

Peritoneal macrophages from normal rats did not phagocytose parasitized erythrocytes in the presence of any of the normal or anti-normal erythrocyte sera. Slight phagocytosis occurred in the presence of foetal calf serum but the maximum phagocytic index recorded was 0·085. Anti- *Babesia* sera did induce phagocytosis (Fig. 1), and the phagocytic index ranged from a minimum of 0·24 for terminal dilutions of antisera to a maximum of 2·35 for the most potent antisera. Some phagocytosis of free parasites was observed but it was not possible to assess this accurately because of the difficulty in identifying such parasites within cells. Phago-
TABLE 1—EFFECT OF ANTISERA ON TIME TO 2 PER CENT PARASITAEMIA IN RECIPIENT RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Assay 1</th>
<th>Assay 2</th>
<th>Assay 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. rodhaini infected r.b.c. antiserum 1:2†</td>
<td>6.5 ± 1.29</td>
<td>12.0 ± 2.16</td>
<td>16.33 ± 2.08§</td>
</tr>
<tr>
<td>B. rodhaini infected r.b.c. antiserum 1:2‡</td>
<td>3.5 ± 0.57</td>
<td>8.5 ± 0.57</td>
<td>14.75 ± 0.95</td>
</tr>
<tr>
<td>B. rodhaini infected r.b.c. antiserum 1:2 primary infection only**†</td>
<td>6.5 ± 1.29</td>
<td>7.75 ± 0.5</td>
<td>6.5 ± 0.71</td>
</tr>
<tr>
<td>B. rodhaini infected r.b.c antiserum 1:2 natural recovery</td>
<td>9.75 ± 0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal r.b.c. antiserum controls</td>
<td>3.25 ± 0.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Normal serum controls 1:2*</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Diampron treated controls 1:2</td>
<td>3.0</td>
<td>—</td>
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</tr>
</tbody>
</table>

Assay 1—performed 5 weeks after primary inoculation.
Assay 2—performed 5 weeks after third inoculation.
Assay 3—performed 5 weeks after sixth inoculation.
* Serum collected at same intervals as for hyperimmunized groups.
† Primary infection drug controlled.
§ No patent infection but immunity to challenge in two out of five rats.

TABLE 2—EFFECT OF ANTISERA ON PARASITAEMIA AND SURVIVAL OF RECIPIENT RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of rats</th>
<th>Mean maximum parasitaemia (%)</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum from uninfected rats 1:2</td>
<td>26</td>
<td>61.4 ± 9.05*</td>
<td>20</td>
</tr>
<tr>
<td>Serum from B. rodhaini infected rats 1:2</td>
<td>24</td>
<td>35.1 ± 19.73*</td>
<td>4</td>
</tr>
</tbody>
</table>

* P < 0.001.

FIG. 1. Erythrophagocytosis of Babesia-infected erythrocytes by normal rat peritoneal macrophages in the presence of anti-Babesia infected erythrocyte serum (× 1200).
cytosis of normal erythrocytes was not induced by any of the sera. Opsonins were detected in all sera in which a protective effect could be demonstrated by assay in rats and increases in protective activity of antisera were paralleled by increases in opsonin titres (Fig. 2). Serum collected at 5 and 20 weeks following a single drug controlled infection contained opsonins to a titre of 1:10 but that taken from rats 5 weeks after they controlled infection unaided had a titre of 1:20 as did serum taken 11 weeks after drug controlled infection.

![Graph](image)

**Fig. 2.** The top half of the figure shows the effect of hyperimmunization with *B. rodhaini*-infected erythrocytes on protective activity of antisera and the level of protective activity at 5, 11 and 20 weeks following infection without hyperimmunization. The protective activity is expressed in days in excess of that in the recipients of the control sera. The bottom half of the figure shows the opsonin titre of these sera and the relationship between titre of opsonins and protective activity. The time scale shown on the bottom of the figure corresponds with the number of inoculations shown on the top.

**DISCUSSION**

The results of passive transfer assay experiments showed that hyperimmunization with *Babesia*-infected erythrocytes produced a corresponding increase in protective activity of antisera. Furthermore in the strain of rats used, anti-erythrocyte antibody played little if any part in the protective activity nor could any significant inhibitory effect, associated with increasing age of the donor rat, be demonstrated. These observations confirm the results of similar experiments performed by Phillips (1969), with the exception that this worker found that immune serum did not usually affect the course and everty of the patent parasitaemia in young rats. In this work the protective activity of hyperimmune antisera was not restricted solely to extension of time to 2 per cent parasitaemia but also resulted in significantly lower peak parasitaemias and a lower mortality. Complete protection and immunity was conferred on two out of five rats receiving the most potent serum. The recipient rats used in this work were somewhat more mature than those of Phillips (1969) and, whilst they were quite susceptible in the absence of immune serum, they were evidently more capable of taking advantage of the protection conferred by hyperimmune serum by activation of their own defense mechanisms. The enhancement of infection by immune serum reported by Ludford (1967) and Roberts (1968) is difficult to reconcile with these results but this work differed in that parasites were not exposed to antibody *in vitro* before inoculation. The suggestion of Roberts (1968) that the effect may be due to suppression of active antibody synthesis by passively transferred antibody is a possible explanation. Subsequent work on *B. rodhaini* infections of mice by Roberts et al. (1972) has shown that the protection conferred by immune serum was more effective against homologous than against heterologous parasites arising from relapses following splenectomy. Thus antigenic variants may have contributed to the failures to demonstrate protection and the use of a frozen reference population of parasites in this work greatly reduced that possibility.

Pipano et al. (1972) found, in *B. bigemina* infections of calves, that drug therapy early in the infection, but not at later stages, severely impaired subsequent immunity. The protective activity of immune serum collected from rats which recovered from infection unaided was significantly greater than that from drug controlled infection and this finding supports the contention of Pipano et al. (1972) that there is a minimum degree of exposure of the host to the parasite for maximum immunity to develop.

Opsonizing antibodies specific for parasitized erythrocytes were detected in all sera in which a protective effect could be demonstrated by passive transfer assay methods. There was a positive relationship between opsonin titre and protective activity and it is suggested that opsonins contributed in large part to this protective effect. They probably acted directly by inducing phagocytosis of many of the infecting parasites, as indicated by the prolongation of prepatent periods, and assisted either directly or indirectly, by allowing the defense mechanisms of the host time to react adequately, in control of subsequent infections. Opsonizing antibodies were detected in serum of monkeys immune to *Plasmodium knowlesi* by Brown et al. (1970) and they considered that opsonins were an important component of the process in which the host controls the parasite. Cohen & Butcher (1970, 1971) and Cohen et al. (1969) described a series of experiments
in which serum from monkeys immune to P. knowlesi inhibited the cyclical proliferation of the parasite in vitro. In later work Butcher & Cohen (1972) maintained that inhibitory antibodies to P. knowlesi provide a consistent index of immune status in monkey malaria and that the schizont agglutinating antibodies detected by Brown & Brown (1965) were less reliable indicators in this respect. However, the relationship between opsonins, inhibitory antibodies and agglutinins is unknown at this stage. Inhibitory antibodies have yet to be demonstrated in babesiosis but their occurrence, together with other antibodies with a possible protective function, such as the agglutinins demonstrated in B. argentina infections of cattle by Curnow (1968), should be considered in assessing the relative importance of opsonizing antibodies in the development and maintenance of acquired immunity.

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REFERENCES
