PROTECTIVE IMMUNITY BETWEEN MALARIA PARASITES AND PIROPLASMS IN MICE

by

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1. INTRODUCTION

Immunity to either malaria or piroplasmosis is usually regarded as being both species and strain specific (see Targett, 1968). However, in recent years there have been a number of reports which suggest that this is not wholly true. In mice, for example, it has been shown that immunity to Plasmodium chabaudi confers a strong resistance to the virulent parasite Plasmodium vinckei (Cox & Voller, 1966; Nussenzweig, Yoeli & Most, 1966; Yoeli et al., 1966 and others) and that immunity to the piroplasm Babesia microti also holds against B. rodhaini (Cox & Young, 1969). These results in themselves could be explained on the basis of the morphological similarities which exist between Plasmodium chabaudi and P. vinckei on one hand and Babesia microti and B. rodhaini on the other, but two subsequent and independent studies have produced results which indicate that this explanation is too simple. Cox & Milar (1968) showed that mice which had recovered from infections with Plasmodium chabaudi were immune to challenge with Babesia rodhaini and Cox (1968) reported, in a preliminary note, that mice which had recovered from infections with B. microti were resistant to challenge with Plasmodium vinckei or P. chabaudi and that this immunity was reciprocal. This paper presents the results of a study on the patterns of cross-immunity between six intra-erythrocytic protozoa in mice, Plasmodium vinckei, P. chabaudi, P. berghei berghei, P. berghei yoelli, Babesia rodhaini and B. microti and a subsequent paper (Cox & Turner, 1970) will consider the antigenic relationships of those same parasites.

2. MATERIALS AND METHODS

The following strains of parasites were used: Plasmodium vinckei (Katanga 52), P. chabaudi (54X), P. berghei berghei (173K), P. berghei yoelli (RCA 17X) Babesia rodhaini (Antwerp) and B. microti (King's 67). All these parasites were maintained by serial passage of infected blood in Alsever's solution every seven days. The inocula used never exceeded $1 \times 10^6$ parasitized red blood cells. The mice in which the parasites were maintained, and which were used for the experiments described in this paper, were female TO Swiss. All the strains of parasites and mice were free from Eperythrozoon coecoides and Haemobartonella muris.

The experimental procedure was to infect mice with one or other of the six parasites, using $1 \times 10^5 - 1 \times 10^6$ parasites, and to check the progress of the infection by taking regular blood films. Mice recovered naturally from Plasmodium chabaudi, P. berghei yoelli and Babesia microti infections but had to be treated with drugs to prevent their deaths from the other infections. Plasmodium vinckei infections were cured with a single dose of chloroquine phosphate at a dose level of 10 mg/100 g body-weight on the fifth day after infection. P. berghei berghei infections were treated in the same way except that several

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doses of the drug had to be given at intervals of 3–4 days and some mice received six such injections. Infections with Babesia rodhaini were cured with a single injection of amicarbalide isethionate (Diampron, May & Baker Ltd) at a dose level of 2 mg/100 g body-weight on the fifth day after infection. All the mice which had recovered from infections were kept until there were no parasites apparent in the peripheral blood. They were challenged with approximately $1 \times 10^6$ parasitized red blood cells containing the homologous or heterologous strain some time later, up to a maximum of 26 weeks. A preliminary series of experiments showed that the inoculation of uninfected blood or treatment with the drugs chloroquine or amicarbalide isethionate had no effect on the challenge infections, and thereafter the controls used were normal uninfected mice. Blood films were taken daily and the parasitaemias in immunized and control animals were compared. The six parasites provided 36 combinations of immunizing and challenge infections and, because of the vast number of animals which would have been involved, not all were examined in the same detail. The actual numbers of animals used are given in Table 1. The criteria of immunity used were reduced parasitaemias during the first seven days of the infection and survival for 30 days in the case of Plasmodium vinckei, P. berghei berghei and Babesia rodhaini. In practice, no animal which survived this 30-day period died later from the infection.

3. RESULTS

The results obtained are summarized in Table 1. These results showed that there was a considerable degree of heterologous immunity between the parasites used and the actual patterns of parasitaemia after challenge are described below.

3.1 Challenge with Plasmodium vinckei

The results obtained after challenge with P. vinckei and the pattern of infection are shown in Fig. 1. In control mice parasites appeared in the blood on the day after infection and reached levels at which 2% of the red blood cells were infected 3.25 days after infection. The parasitaemias increased in a near logarithmic manner until the mice died on the seventh day after infection with parasitaemias of between 70 and 80%.

P. vinckei/P. vinckei. Twelve mice were initially infected with P. vinckei and challenged with the homologous parasite 7–10 weeks later. No infections occurred.

P. chabaudi/P. vinckei. Thirty mice were initially infected with P. chabaudi and challenged with P. vinckei 7–10 weeks later. Infections developed in 12. In five the parasitaemias were low level and transient, two developed high parasitaemias and recovered while five died 7–10 days after challenge. In the animals which died, the parasitaemias were similar to those in control mice.

P. b. berghei/P. vinckei. Twenty mice were initially infected with P. berghei berghei and challenged with P. vinckei 4–9 weeks later. Infections developed in 17. The patterns of parasitaemias are shown in Fig. 1. The parasitaemias rose in exactly the same way as in control animals for the first five days after infection, but thereafter declined in 14 mice and rose in the other three which died seven, eight and nine days after challenge.

P. b. yoelii/P. vinckei. Twelve mice were initially infected with P. berghei yoelii and challenged with P. vinckei 5–13 weeks later. Infections developed in all the mice. The patterns of parasitaemias are shown in Fig. 1. In seven mice the parasitaemias rose in the same way as in control animals for the first five days of the infection and then more slowly until day 7; thereafter the parasitaemias declined. In the remaining five mice the parasitaemias did not decline and the mice died on days 6, 7 (2 mice), 13 and 14 after challenge.
Babesia rodhaini/Plasmodium vinckei. Seventeen mice were initially infected with B. rodhaini and challenged with P. vinckei 4-6 weeks later. Infections developed in eight. In six of these the parasitaemias were low level and transient and in one the parasitaemia rose to a high peak seven days after infection and then fell. The remaining mouse died seven days after challenge.

Babesia microti/Plasmodium vinckei. Twenty-one mice were initially infected with B. microti and challenged with P. vinckei 6-14 weeks later. Infections developed in 12. In seven the parasitaemias were low level and transient, in two the infections rose as in the controls until the fifth day after infection and then declined. Three mice died on the seventh, tenth and twelfth day after challenge with parasitaemias resembling those in the control animals.

3.2 Challenge with Plasmodium chabaudi

The results obtained after challenge with P. chabaudi and the pattern of infection are shown in Fig. 2. In control mice parasites appeared in the blood on the day after infection and reached levels at which 2% of the red blood cells were infected 3-25 days after infection. The parasitaemias increased in a near logarithmic manner until the fourth day after infection and then continued to rise less rapidly to reach peaks at which about 40% of the red blood cells were infected on the seventh day after infection. Thereafter the parasitaemias gradually declined and parasites disappeared from the blood by the twentieth day after infection.

P. chabaudi/P. chabaudi. Sixteen mice were initially infected with P. chabaudi and challenged with the homologous parasite 6-13 weeks later. Infections developed in two. In both cases the parasitaemias were low level and transient.

P. vinckei/P. chabaudi. Twenty mice were initially infected with P. vinckei and challenged with P. chabaudi 4-10 weeks later. Infections developed in 16. In nine the parasitaemias were low level and transient, in six the parasitaemias were similar to those in the control animals and in one the peak of parasitaemia was delayed until the twelfth day after challenge.

P. b. berghei/P. chabaudi. Nine mice were initially infected with P. b. berghei and challenged with P. chabaudi 5-17 weeks later. Infections developed in eight. The patterns of parasitaemia are shown in Fig. 2. In three mice the parasitaemias were very low, in one the parasitaemia was higher but lower than in the controls and in four the parasitaemias approached those in the control animals.

P. yoelii/P. chabaudi. Twelve mice were initially infected with P. b. yoelii and challenged with P. chabaudi 5-10 weeks later. Infections developed in all the mice. The patterns of parasitaemia are shown in Fig. 2. In four mice the parasitaemias were low level but in the remainder approached those in the control animals. Two mice died on days 7 and 9 after challenge.

Babesia rodhaini/Plasmodium chabaudi. Fifteen mice were initially infected with B. rodhaini and challenged with P. chabaudi 4-10 weeks later. Infections developed in 14. The patterns of parasitaemia are shown in Fig. 2. In five animals the parasitaemias were considerably lower than in the control animals. In eight of the remaining mice the parasitaemias were similar to those in control animals and in one the peak parasitaemia was similar but delayed until the tenth day after challenge.

Babesia microti/Plasmodium chabaudi. Twenty-four mice were initially infected with B. microti and challenged with P. chabaudi 6-12 weeks later. Infections developed in 22. The patterns of parasitaemia are shown in Fig. 2. In 18 the parasitaemias were considerably lower than in control animals and the peaks of parasitaemia were delayed until the ninth day after challenge (see Fig. 2). In the remaining six mice the parasitaemias did not differ significantly from those in the control animals.
3.3 Challenge with Plasmodium berghei berghei

The results obtained after challenge with P. b. berghei and the patterns of infection are shown in Fig. 3. In control mice parasites appeared in the blood on the second day after infection and reached levels at which 2% of the red blood cells were infected six days after infection. The parasitaemias increased until about 50% of the red blood cells were infected 18 days after infection and thereafter oscillated about this level until the mice died between the twentieth and thirtieth day after infection.

P. b. berghei/P. b. berghei. Ten mice were initially infected with P. berghei and challenged with the homologous parasite 3-6 weeks later. Infections developed in five. In three the parasitaemias were low and transient but in the remaining two they approached the levels seen in the control animals.

P. vinckei/P. b. berghei. Fifteen mice were initially infected with P. vinckei and challenged with P. b. berghei 6-9 weeks later. Infections developed in all cases. In 14 the parasitaemias were higher than in control animals and all these mice died. (See Fig. 3)

P. chabaudi/P. b. berghei. Twenty-four mice were initially infected with P. chabaudi and challenged with P. b. berghei 4-13 weeks later. Infections developed in all cases. In six the levels of parasitaemia were slightly lower than those in control animals but in the remaining 18 they were higher. (See Fig. 3)

P. b. yoelii/P. b. berghei. Twelve mice were initially infected with P. b. yoelii and challenged with P. b. berghei 5-9 weeks later. Infections developed in all mice. In four the parasitaemias were low and transient and in eight they did not differ from those in control animals. (See Fig. 3)

Babesia rodhaini/Plasmodium b. berghei. Eleven mice were initially infected with B. rodhaini and challenged with P. b. berghei 3-10 weeks later. Infections developed in all cases. The levels of parasitaemia were similar to those in control animals and all the mice challenged died between the twelfth and twenty-sixth day after infection. (See Fig. 3)

Babesia microti/Plasmodium b. berghei. Thirty mice were initially infected with B. microti and challenged with P. b. berghei 4-14 weeks later. Infections developed in all cases. In 28 mice the levels of parasitaemia were as high as or higher than in control animals and the mice died between the twelfth and thirty-first day after challenge. (See Fig. 3) In two mice the levels of parasitaemia were lower than in control animals, never rising above 3% and the infected mice did not die.

3.4 Challenge with Plasmodium berghei yoelii

The results obtained after challenge with P. b. yoelii and the patterns of infection are shown in Fig. 4. In control mice parasites appeared in the blood on the second day after infection and reached levels at which 2% of the red blood cells were infected six days after infection. The parasitaemias increased until about 14% of the red blood cells were infected 12 days after infection and thereafter declined, disappearing altogether by the seventeenth day.

P. b. yoelii/P. b. yoelii. Ten mice were initially infected with P. b. yoelii and challenged with the homologous strain 5-10 weeks later. No infections developed.

P. vinckei/P. b. yoelii. Ten mice were initially infected with P. vinckei and challenged with P. b. yoelii five weeks later. Infections developed in all cases. (See Fig. 4) The parasitaemias rose in exactly the same way as in control animals for the first seven days after challenge to reach a peak of 4% but thereafter declined, disappearing altogether by the eleventh day after challenge.
P. chabaudi/P. b. yoelii. Twelve mice were initially infected with P. chabaudi and challenged 3-26 weeks later with P. b. yoelii. Infections developed in all animals. In four the levels of parasitaemia were low and transient, in six they resembled those in the control animals and in two they resembled those in control animals except that the peaks of parasitaemia occurred about five days later.

P. b. berghei/P. b. yoelii. Twelve mice were initially infected with P. b. berghei and challenged 3-13 weeks later with P. b. yoelii. Infections developed in six. In one of these the parasitaemia never rose above 2% while in the remaining five the parasitaemias resembled those in the control animals.

Babesia rodhaini/Plasmodium b. yoelii. Eleven mice were initially infected with B. rodhaini and challenged 4-11 weeks later with P. b. yoelii. All became infected. (See Fig. 4) The parasitaemias rose in the same way as in control animals for the first 10 days after challenge, then fell slowly to rise again until the eighteenth day after challenge and thereafter declined.

Babesia microti/Plasmodium b. yoelii. Fifteen mice were initially infected with B. microti and challenged with P. b. yoelii 8-21 weeks later. All became infected. (See Fig. 4) The parasitaemias rose in the same way as in control animals, except that they were somewhat higher, to reach peaks on the twelfth day after challenge. Thereafter the parasitaemias declined and disappeared by the twenty-second day after challenge.

3.5 Challenge with Babesia rodhaini

The results obtained after challenge with B. rodhaini and the patterns of infection are shown in Fig. 5. In control mice, parasites appeared in the blood on the day after infection and reached levels at which 2% of the red blood cells were infected three days after infection. The parasitaemias increased in a near logarithmic manner until the mice died on the 7-8th day after infections with parasitaemias between 70 and 80%.

B. rodhaini/B. rodhaini. Twelve mice were initially infected with B. rodhaini and challenged with the homologous parasite six weeks later. Infections developed in six, but the levels of parasitaemia never rose above 0.1%.

Plasmodium vinckei/Babesia rodhaini. Twelve mice were initially infected with P. vinckei and challenged with B. rodhaini 3-6 weeks later. Infections developed in eight. The parasitaemias were low level and transient.

Plasmodium chabaudi/Babesia rodhaini. Twenty-two mice were initially infected with P. chabaudi and challenged with B. rodhaini 7-23 weeks later. Infections developed in 16. In six mice the infections were low level and transient, in one the parasitaemia rose to the same level as in the control group, but the animal recovered, and in nine the parasitaemias were similar to those in the control group and the mice died between the sixth and tenth day after challenge.

Plasmodium b. berghei/Babesia rodhaini. Fifteen mice were initially infected with P. b. berghei and challenged with B. rodhaini 4-6 weeks later. Infections developed in 11. In two of these the parasitaemias were low level and transient, in two the parasitaemias were originally low but gradually rose reaching, 25 days after challenge, about 50% and then suddenly declined. In two the parasitaemias were similar to those in the control group but the mice recovered. The remaining five mice all had parasitaemias resembling those in the control group and died on the sixth, seventh, eighth, ninth and twenty first days after challenge.
Plasmodium b. yoelii/Babesia rodhaini. Twelve mice were initially infected with P. berghei yoelii and challenged with B. rodhaini 5-10 weeks later. All developed infections. (See Fig. 5) In two mice the parasitaemias were low level and transient and in the remainder the parasitaemias were similar to those in the control group until the fourth day after challenge when they ceased to rise so rapidly and reached peaks at which 30% of the red blood cells were infected on the seventh day. Thereafter the parasitaemias began to decline and parasites had disappeared from the blood by the seventeenth day. Three mice died between the sixth and eighth day after challenge.

B. microti/B. rodhaini. Twenty mice were initially infected with B. microti and challenged with B. rodhaini 4-9 weeks later. Infections developed in 14. In 12 of these mice the parasitaemias were low and never reached a level of more than 2%. In the remaining two mice the parasitaemias rose to about 40% seven days after infection and then declined.

3.6 Challenge with Babesia microti

The results obtained after challenge and the pattern of infection are shown in Fig. 6.

In control mice parasites appeared in the blood on the second day after infection and reached levels at which 2% of the red blood cells were infected 3.5 days after infection. The parasitaemias increased to peaks at which about 55% of the red blood cells were infected on the eleventh day after infection and then slowly declined until no parasites were seen in the blood 25 days after infection.

B. microti/B. microti. Ten mice were initially infected with B. microti and challenged with the homologous parasite seven weeks later. Infections developed in seven but did not rise above a level of 0.1%.

Plasmodium vinckei/Babesia microti. Eleven mice were initially infected with P. vinckei and challenged with B. microti 4-10 weeks later. Infections developed in three but in two only an occasional parasite was seen.

Plasmodium chabaudi/Babesia microti. Nineteen mice were initially infected with P. chabaudi and challenged with B. microti 5-11 weeks later. Infections developed in eight mice but only an occasional parasite was seen and in one mouse there was no patent parasitaemia until the twenty-seventh day after infection.

Plasmodium b. berghei/Babesia microti. Sixteen mice were initially infected with P. b. berghei and challenged with B. microti 6-7 weeks later. Infections developed in 11. In six the parasitaemias were low level and transient, in one the parasitaemia rose to a peak 17 days after infection and then declined and in two the infections resembled those in the control group. Two mice actually died from the infection 21 and 25 days after infection.

Plasmodium b. yoelii/Babesia microti. Twelve mice were initially infected with P. b. yoelii and challenged 5-7 weeks later with B. microti. All became infected. (See Fig. 6) In four mice the parasitaemias were low level and transient, in three they rose to low peaks of about 10% on day 10 and in five the parasitaemias resembled those in the control group. Two mice died on the eleventh day after challenge.

B. rodhaini/B. microti. Twelve mice were initially infected with B. rodhaini and challenged with B. microti seven weeks later. Infections developed in nine but in no case did the parasitaemia rise above a level of 1%.

3.7 Attempted immunization with serum from infected mice

In order to eliminate the possibility that some factor present in the serum of infected animals might be immunizing the mice against challenge infections, blood was taken from
mice at the height of parasitaemia, filtered through an 0.45 μm filter and injected into uninfected mice. This was repeated for all parasites and in no case did this procedure result in any protection on subsequent challenge with the homologous or heterologous species.

4. DISCUSSION

The six parasites used in this investigation were chosen for the following reasons. Firstly, they had all been maintained in mice for some time and always gave rise to reproducible infections in these animals. Thus their use avoided any complications due to changes which might have occurred on transfer from one host to another. Secondly, they represented three comparable pairs of morphologically similar parasites, each of which gave rise to a different kind of infection; one virulent and one benign. Thus Plasmodium vinckeii and P. chabaudi were morphologically similar although P. vinckeii killed mice in about seven days while animals infected with P. chabaudi nearly always recovered. Similarly P. b. berghei and P. b. yoelii and Babesia rodhaini and B. microti constituted morphological pairs in which the first named was the virulent form. These six parasites, then, provided the opportunity to study homologous immunity and various degrees of heterologous immunity ranging from what amounted to intraspecific immunity through intrageneric to suprageneric immunity. In order to obtain as much information as possible from a relatively small number of animals the mice were challenged randomly after recovery in such a way that infections with various parasites could be compared. This accounts for the variations in the periods between infection and challenge. The shortcomings of this approach were appreciated but limited animal accommodation permitted no other possibility. As things transpired, however, the period between the initial infection and challenge was irrelevant in the context of the present study.

This investigation occupied about a year and during this time no signs of Eperythrozoon coccoides or Haemobartonella muris were apparent, despite continual surveillance and routine splenectomies. Similarly, the inoculation of uninfected blood or the serum from infected animals gave no protection to challenge whatsoever and thus it is concluded that the results obtained were not due to contamination with some other infective organism. This conclusion is confirmed by the duration of the immunity observed which exceeds the short-lived resistance induced by such organisms as Eperythrozoon (see Voller & Bidwell, 1968).

The results obtained indicate that immunity to the homologous parasite is strong and thus confirm observations made previously (Cox, 1966; Cox & Voller, 1966 and Cox & Young, 1969). A considerable degree of heterologous immunity also exists and in most cases this extends to the heterologous member of the pair and to other species and genera. A number of observers have recorded immunity between Plasmodium chabaudi and P. vinckeii (Cox & Voller, 1966; Nussenzweig et al., 1966; Yoelii et al., 1966 and others). Immunity has also been recorded between P. vinckeii and P. chabaudi (Cox & Voller, 1966) and between Babesia microti and B. rodhaini and vice versa (Cox & Young, 1969). Several workers have reported the absence of immunity between Plasmodium chabaudi and P. b. berghei and these include Cox & Voller (1966), Nussenzweig et al. (1966), Yocli et al. (1965) and Cox & Milar (1968). Demina, Glazunova & Chouksina (1969) have reported the absence of immunity between P. b. yoelii and P. b. berghei. Apart from the preliminary note by Cox (1968) there is only a single report of suprageneric immunity and this is by Cox & Milar (1968) who showed that mice which had recovered from infections with P. chabaudi were immune to challenge with Babesia rodhaini.

The degree of heterologous immunity is best seen by reference to Tables 1, 2 and 3, from which it is apparent that recovery from all six parasites results in some degree of immunity to the homologous strains, the heterologous, although similar, strains and to heterologous species. The results are best analysed by considering the challenge infections. All six parasites protected the majority of mice against Plasmodium vinckeii, which was a particularly easy infection to study as all the control animals died. The most unexpected result was the high degree of protection afforded by the two piroplasms. Another interesting result was
seen in mice which had recovered from infections with *P. b. berghei*. As has been pointed out above, previous observations (Cox & Voller, 1966) failed to reveal any immunity between *P. b. berghei* and *P. vinckeii* and the different results obtained in the present study are probably due to the use of different lines of the same strain and a difference in technique. In order to maintain parity between all the parasites used the inocula were maintained at $1 \times 10^6$ or less which is smaller than the challenge dose formerly used. Fig. 1 clearly shows that in mice which had recovered from *P. b. berghei* or *P. b. yoelii* infections the parasitaemias rose in exactly the same way as in control animals until the fifth day after challenge when they began to decline. It seems probable that the process of immunity elicited by the other parasites is different from that elicited by *P. b. berghei* or *P. b. yoelii*, for in the others, the challenge infection never really became established, suggesting an immune mechanism operating from the time of challenge, whereas after *P. b. berghei* or *P. b. yoelii* infections the immune mechanism only became manifest on the fifth day after challenge. In the earlier experiments (Cox & Voller, 1966) the higher challenge doses used, and possibly also the male mice, never allowed this late immune response to occur.

All six parasites also protected at least some mice against *P. chabaudi* but protection was least in mice previously infected with *P. b. berghei* or *P. b. yoelii*. These particular results are in general agreement with those reported previously. As far as initial infections with piroplasms are concerned, the degree of protection afforded against *P. chabaudi* was less than that against *P. vinckeii*. Fig. 2 shows that the patterns of parasitaemia following challenge with *P. chabaudi* are basically similar, although the intensity of the infections varies, suggesting a similarity in the immune mechanism.

There was practically no heterologous immunity against *P. b. berghei*, only *P. b. yoelii* being protective at all. The absence of immunity afforded by *P. vinckeii* is in agreement with the observations of Cox & Voller (1966). The lack of immunity following infections with *P. chabaudi* is in agreement with observations recorded by Cox & Voller (1966), Nussenzweig et al. (1966), Yoelli et al. (1966) and Cox & Milar (1968). Cox & Milar (1968) noted a reduction in the level of parasitaemia which was seen in some mice in the present study. Demina et al. (1969) have also recorded a lack of immunity between *P. b. yoelii* and *P. b. berghei*. Fig. 3 shows clearly that far from protecting against *P. b. berghei* previous infections with *P. vinckeii*, *P. chabaudi*, Babesia rodhaini and *B. microti* resulted in higher parasitaemias than in control animals.

Heterologous immunity to *Plasmodium b. yoelii* was more marked than that to *P. b. berghei* in mice previously infected with *P. vinckeii* or *P. chabaudi* but less than that to other parasites. In mice which had recovered from infections with *P. vinckeii* the challenge infections rose unchecked until the seventh day when the parasitaemias began to decline, suggesting an immune mechanism which became effective late after challenge. No immunity was elicited by the piroplasms Babesia rodhaini or *B. microti* which actually enhanced the challenge infections, as can be seen by reference to Fig. 4.

There was a considerable degree of heterologous immunity against *B. rodhaini* and the greatest protection was afforded by *Plasmodium vinckeii*. This immunity was as strong as, if not stronger than, the homologous immunity. The least immunity was afforded by *P. b. berghei*. In mice which had recovered from infections with *P. b. yoelii* the immunity became apparent about five days after challenge, as can be seen by reference to Fig. 5.

Heterologous immunity to Babesia microti was similar to that to *B. rodhaini*; a very strong immunity resulted from infection with *Plasmodium vinckeii* and the least immunity followed infections with *P. b. berghei*. After infections with *P. b. yoelii* the patterns of parasitaemia after challenge were similar but lower than in control animals. (See Fig. 6)

In summarizing these results it is apparent that the greatest degree of heterologous immunity exists against *Plasmodium vinckeii*, Babesia rodhaini and *B. microti*, less against *Plasmodium chabaudi* and *P. b. yoelii* and least against *P. b. berghei*. As a working hypothesis
it is suggested that a certain degree of homologous and heterologous immunity is induced by all these parasites and that the breakdown of this immunity is dependent on the ability of the parasite to evade this immune response. This ability may well be correlated with the degree of antigenic variation exhibited by these parasites. *P. vinckei* and *Babesia rodhaini* are both virulent and kill their hosts within about seven days. They have both been maintained in mice for a number of years and are now probably stable variants. *B. microti* has been carefully passaged at regular intervals since its isolation over two years ago so is probably also a stable variant. *Plasmodium chabaudi* and *P. b. yoelii* have relatively long periods of patency and thus every opportunity to undergo antigenic variation but the infections are overcome within a month, suggesting a limited range of antigenic potential. *P. b. berghei* is different. The course of infection, under the conditions of this investigation, tends to be long and the parasitaemia may remain at an elevated level for over 30 days before the mouse eventually dies, and this suggests an active process of antigenic variation in which the parasite eventually defeats the immune response of the host. It is suggested that it is this capacity for antigenic variation that enables *P. b. berghei* to survive both homologous and heterologous immunity. The fact that immunity induced by *P. b. berghei* against *P. vinckei*, *Babesia rodhaini* and *B. microti* is not reciprocal suggests that immunity is a function of the parasite and not an inadequacy on the part of the host's immune response. Cox & Milar (1968) have suggested that antigens released into the plasma of infected animals provide the basis of the heterologous immunity between *Plasmodium chabaudi* and *Babesia rodhaini* and that the lack of immunity between *Plasmodium chabaudi* and *P. b. berghei* is due to the selection of "immunity resistant" strains. Serum soluble antigens were not studied in this investigation, but the antigenic nature of the six parasites used has been studied by means of a fluorescent antibody technique (Cox & Turner, 1970). The results obtained showed that all the parasites possessed antigens in common and the presence of these common antigens could form a basis for heterologous immunity. However, the actual levels of antibody titres, which correspond with the degree of antigenic similarity, cannot be correlated with the degree of protective immunity. The conclusion must therefore be that common antigens, either bound or serum soluble, enable the host to recognize the invading parasites and to react against them. This would account for both the failure of challenge infections to establish or for the decline of infections after they had paralleled the control ones for about five days. Antigenic variation, or the "selection of immunity resistant strains", if this is different, must account for failures of the immune response. Heterologous immunity cannot be dismissed as a non-specific (and therefore non-immunological) reaction because if this were the case the same degree of "immunity" should have been induced by all parasites to all challenge infections. The absence of immunity in some cases emphasizes the specificity of the immune response.

There are several significant features of these results. Firstly, there is the heterologous immunity itself and its possible implications for the study of homologous immunity. Secondly, there is the possibility that rodents collected in the field may be resistant to an infection because of prior exposure to another unrelated one. Finally, there must exist the possibility that humans exposed to the piroplasms of rodents or other animals may thereby acquire an immunity to malaria.
<table>
<thead>
<tr>
<th>Immunizing infection</th>
<th>Challenge infection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasmodium vinckei</td>
<td>P. chabaudi</td>
</tr>
<tr>
<td>Plasmodium vinckei</td>
<td>12/12</td>
<td>14/20</td>
</tr>
<tr>
<td>P. chabaudi</td>
<td>25/30</td>
<td>16/16</td>
</tr>
<tr>
<td>P. b. berghei</td>
<td>17/20</td>
<td>4/9</td>
</tr>
<tr>
<td>P. b. yoelii</td>
<td>7/12</td>
<td>4/12</td>
</tr>
<tr>
<td>Babesia rodhaini</td>
<td>16/17</td>
<td>6/15</td>
</tr>
<tr>
<td>B. microti</td>
<td>18/21</td>
<td>18/24</td>
</tr>
</tbody>
</table>
### TABLE 2. THE PERCENTAGE OF MICE PROTECTED AGAINST CHALLENGE WITH HETEROLOGOUS PARASITES

<table>
<thead>
<tr>
<th>Challenge infection</th>
<th>Percentage of mice protected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All heterologous strains</td>
<td>Heterologous strains excluding morphologically similar forms</td>
</tr>
<tr>
<td>Plasmodium vinckeii</td>
<td>83</td>
<td>81</td>
</tr>
<tr>
<td>P. chabaudi</td>
<td>58</td>
<td>53</td>
</tr>
<tr>
<td>P. b. berghei</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>P. b. yoelii</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>Babesia rodhaini</td>
<td>79</td>
<td>72</td>
</tr>
<tr>
<td>B. microti</td>
<td>84</td>
<td>81</td>
</tr>
</tbody>
</table>

### TABLE 3. THE PERCENTAGE OF MICE PROTECTED BY HETEROLOGOUS PARASITES

<table>
<thead>
<tr>
<th>Immunizing infection</th>
<th>Percentage of mice protected</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All heterologous strains</td>
<td>Heterologous strains excluding morphologically similar forms</td>
</tr>
<tr>
<td>Plasmodium vinckeii</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>P. chabaudi</td>
<td>57</td>
<td>47</td>
</tr>
<tr>
<td>P. b. berghei</td>
<td>68</td>
<td>70</td>
</tr>
<tr>
<td>P. b. yoelii</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>Babesia rodhaini</td>
<td>51</td>
<td>41</td>
</tr>
<tr>
<td>B. microti</td>
<td>53</td>
<td>42</td>
</tr>
</tbody>
</table>
FEMALE TO SWISS MICE, WHICH WERE FREE FROM INFECTIONS WITH EPERYTHROZOON OR HAEMOBARTONELLA, WERE INFECTED WITH ONE OF THE FOLLOWING SIX PARASITES: PLASMODIUM VINCKEI, P. CHABAUDI, P. BERGHEI BERGHEI, P. B. YOELII, BABESIA RODHAINI OR B. MICROTI. THE MICE WERE ALLOWED TO RECOVER NATURALLY, OR THE INFECTIONS WERE TERMINATED WITH DRUGS, AND THEN CHALLENGED WITH THE HOMOLOGOUS OR HETEROLOGOUS STRAINS. ALL THE MICE WERE IMMUNE TO CHALLENGE WITH THE HOMOLOGOUS PARASITES AND ALL SHOWED SOME DEGREE OF IMMUNITY TO CHALLENGE WITH HETEROLOGOUS SPECIES. ALTHOUGH INFECTIONS WITH PLASMODIUM B. BERGHEI INDUCED IMMUNITY AGAINST THE MAJORITY OF THE OTHER PARASITES, THIS IMMUNITY WAS NOT RECIPROCAL AND IMMUNITY TO P. B. BERGHEI WAS INDUCED ONLY BY THE HOMOLOGOUS PARASITE. IT IS SUGGESTED THAT ALL THESE SIX PARASITES INDUCE IMMUNE RESPONSES WHICH EXTEND TO THE HETEROLOGOUS SPECIES BUT THAT P. B. BERGHEI IS ABLE TO EVADE THIS RESPONSE PROBABLY BY UNDERGOING A SERIES OF ANTIGENIC VARIATIONS.

RESUME


ACKNOWLEDGEMENTS

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REFERENCES

Cox, F. E. G. (1966) *Parasitology, 56*, 719-732


Cox, F. E. G. & Turner, S. A. (1970) Antigenic relationships between the malaria parasites and piroplasms of mice as determined by the fluorescent antibody technique, WHO/MAL/70.115 (Mimeographed document)


The purpose of the WHO/MAL series of documents is threefold:

(a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;

(b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;

(c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

It should be noted that the summaries of unpublished work often represent preliminary reports of investigations and therefore such findings are subject to possible revision at a later date.

The mention of manufacturing companies or of their proprietary products does not imply that they are recommended or endorsed by the World Health Organization.
Fig. 1. Graphs showing the patterns of parasitaemia in 12 mice infected with *Plasmodium vinckei*, in 17 mice which had recovered from *P. b. berghei* infections and which became infected on challenge with *P. vinckei* and in 12 mice which had recovered from *P. b. yoelii* infections and which became infected on challenge with *P. vinckei*. In this and all subsequent figures some of the points have been left out to avoid confusion.

- - *P. vinckei* controls
- - *P. b. berghei/P. vinckei*
- - *P. b. yoelii/P. vinckei*
Fig. 2. Graphs showing the patterns of parasitaemia in 12 mice infected with *Plasmodium chabaudi*, in 8 mice which had recovered from infections with *P. b. berghei* and which became infected on challenge with *P. chabaudi*, in 12 mice which recovered from infections with *P. b. yoelii* and which became infected on challenge with *P. chabaudi*, in 14 mice which had recovered from infections with *Babesia rodhaini* and which became infected on challenge with *Plasmodium chabaudi* and in 22 mice which had recovered from infections with *Babesia microti* and which became infected on challenge with *Plasmodium chabaudi*.

- O—O *P. chabaudi* controls
- ■—■ *P. b. berghei/P. chabaudi*
- □—□ *P. b. yoelii/P. chabaudi*
- ▲—▲ *B. rodhaini/P. chabaudi*
- △—△ *B. microti/P. chabaudi*
Fig. 3. Graphs showing the patterns of parasitaemia in 12 mice infected with Plasmodium b. berghei, in 15 mice which had recovered from infections with P. vinckei and which became infected on challenge with P. b. berghei, in 24 mice which had recovered from infections with P. chabaudi and which became infected on challenge with P. b. berghei, in 12 mice which had recovered from infections with P. b. yoelii and which became infected on challenge with P. b. berghei, in 11 mice which had recovered from infections with Babesia rodhaini and which became infected on challenge with Plasmodium b. berghei and in 30 mice which had recovered from infections with Babesia microti and which became infected on challenge with Plasmodium b. berghei.

- ■ P. b. berghei controls
- ● P. vinckei/P. b. berghei
- ○ P. chabaudi/P. b. berghei
- □ P. b. yoelii/P. b. berghei
- ▲ B. rodhaini/P. b. berghei
- △ B. microti/P. b. berghei
Fig. 4. Graphs showing the patterns of parasitaemia in 12 mice infected with *Plasmodium b. yoelii*, in 10 mice which had recovered from infections with *P. vinckei* and which became infected on challenge with *P. b. yoelii*, in 11 mice which had recovered from infections with *Babesia rodhaini* and which became infected on challenge with *Plasmodium b. yoelii* and in 15 mice which had recovered from infections with *Babesia microti* and which became infected on challenge with *Plasmodium b. yoelii*. 

Per cent. red blood cells infected

Days after challenge
Fig. 5. Graphs showing the patterns of parasitaemia in 12 mice infected with *Babesia rodhaini*, and in 12 mice which had recovered from infections with *Plasmodium b. yoelii* and which became infected on challenge with *Babesia rodhaini*.

- ▲▲ B. rodhaini controls
- □□ P. b. yoelii/B. rodhaini
Fig. 6. Graphs showing the patterns of parasitaemia in 12 mice infected with *Babesia microti* and in 12 mice which had recovered from infections with *Plasmodium b. yoelii* and which became infected on challenge with *Babesia microti*

△△ *B. microti* controls

□□ *P. b. yoelii/B. microti*