The Effect of Rowson–Parr Virus on the Severity of Malaria in Mice

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SUMMARY

Rowson–Parr virus (RPV), which causes lymphoma in Balb/c mice and also depresses the splenic haemolytic plaque-forming cell response to sheep erythrocytes, greatly exacerbates infections caused by two murine *Plasmodium* species. One of these, *Plasmodium vinckei chabaudi*, infects mature erythrocytes and the other, *Plasmodium berghei yoelii*, infects mainly reticulocytes. Mice infected with RPV and either *Plasmodium* showed a similar degree of reticulocytosis to those which received the *Plasmodium* alone. Antiplasmodial antibody was much higher in mice which received *Plasmodium* alone than in those also infected with RPV.

INTRODUCTION

Two murine lymphomagenic viruses, the Rowson–Parr virus (RPV) (Rowson & Parr, 1970) and ULV, a virus isolated from a urethane-induced leukaemia (Salaman & Flocks, 1964), enhance infections caused by *Plasmodium berghei yoelii* (*P.b.y.*) in Balb/c mice (Salaman & Wedderburn, 1969; Salaman, Wedderburn & Bruce-Chwatt, 1969). Combined infections with *P.b.y.* and either ULV or RPV lead to the death of the mice with fulminating parasitaemia. Rowson–Parr virus and ULV depress immune responses to sheep erythrocytes (Wedderburn, 1969; Carter *et al.* 1970) and it seemed possible that their effect on malarial infection was due to suppression of the immune response to *Plasmodium* spp. However, *P.b.y.* preferentially invades reticulocytes, and changes in the balance of mature red cells and reticulocytes are often more important than the immune status of the host in limiting infections (Spira, Golenser, Zuckerman & Neiss, 1972; Cox, 1974). Here we compare the effects of RPV on infections with *P.b.y.* and with *Plasmodium vinckei chabaudi* (*P.v.c.*), a parasite which preferentially invades mature red cells.

METHODS

Female Balb/c mice, maintained by brother–sister mating, were used.

Plasmodium berghei yoelii (17×) (*P.b.y.*) and *Plasmodium vinckei chabaudi* (54×) (*P.v.c.*) were used: 10⁶ parasitized erythrocytes from an infected mouse were injected intraperitoneally.

Mice were infected with Rowson–Parr virus (RPV) by intravenous injection of 0.1 ml citrated plasma containing 10³ to 10⁴ ID₅₀ (median infective dose).

Antibody levels were estimated using an indirect fluorescent antibody technique (Voller

Table 1. *Mortality of mice given Rowson-Parr virus (RPV) at intervals between 15 days before and 7 days after infection with Plasmodium berghei yoelii*

Each group contained 8 mice.

Group	Day of RPV	Deaths
1	-15	0
2	-12	0
3	-8	0
4	-6	3
5	-5	7
6	-4	7
7	-1	4
8	+3	4
9	+7	0
10	-	0

& O'Neill, 1971). The antigen consisted of washed red blood cells infected with the homologous parasite. The conjugate was commercially prepared rabbit anti-mouse Ig (Miles Laboratories).

Haemolytic plaque-forming cells (PFC) were determined by the Jerne technique with minor modifications (Salaman & Wedderburn, 1968).

RESULTS

Preliminary experiments

Balb/c mice 7 to 12 weeks old all recovered from infection with *P.b.y.* after about 17 days; 8 to 20 % of the red blood cells were infected at the peak. Mice less than 8 weeks old almost invariably died from infection with *P.v.c.*, but 50 % or more of 11 to 12-week-old mice recovered. In the survivors, the pattern of infection was similar to that seen with *P.b.y.* When given RPV between 4 days before and 4 days after either species of *Plasmodium*, most mice died.

Effects of Rowson-Parr virus on the course of the disease caused by Plasmodium berghei yoelii

Experiment 1. Nine groups of 8 mice 12 weeks old were infected with *P.b.y.* on day 0, and with RPV at various times from days -15 to +7 (Table 1). No mouse given RPV 8 or more days before infection with *P.b.y.* died, nor did any given the virus 7 days afterwards. Comparable groups of mice were injected with sheep erythrocytes (SE) on day 0 and with RPV at various times; haemolytic plaque-forming cells (PFC) in the spleen were estimated 4 days after SE injection. The greatest depression of the PFC response and the highest mortality occurred when RPV was injected 5 or 4 days before either SE or *P.b.y.* (Fig. 1).

Experiment 2. Two groups of 6 mice, 7 to 8 weeks old, were infected with *P.b.y.*, or with *P.b.y.* and RPV together. Daily blood smears showed that reticulocytosis was similar in the two groups (Fig. 2). In the group infected with both agents, parasitaemias continued to rise until all the mice died.

Experiment 3. Two groups of 6 mice 7 to 8 weeks old were infected with *P.b.y.* and with *P.b.y.* and RPV together, respectively. Mice given *P.b.y.* only had anti-malarial antibody titres (estimated by immunofluorescence) of 1:640 by day 18, while mice receiving *P.b.y.* and RPV never developed titres higher than 1:40 (Fig. 3).

Fig. 1. Haemolytic plaque-forming cells (PFC) to sheep erythrocytes (SE) and mortality of mice given Rowson-Parr virus (RPV) at various times relative to either erythrocytes or *Plasmodium berghei yoelii* (*P.b.y.*). ○—○, Mortality of mice infected with *P.b.y.* on day 0; ●—●, PFC/spleen of mice injected with SE on day 0; ---, PFC/spleen of control mice (SE on day 0, no RPV or *P.b.y.*).

Effect of Rowson-Parr Virus on the course of the disease caused by Plasmodium vinckei chabaudi

Eleven mice aged 12 weeks received *P.v.c.* and RPV together and 8 mice received *P.v.c.* alone. All of the former group died; the mean survival time (MST) was 10.7 days. In the latter group only 4 mice died, with an MST of 10.5 days. Percentages of parasitaemias were similar in both groups until day 7, when about 50% of the red cells were infected, after which they declined rapidly in survivors and rose to 80 to 90% in the mice which died (Fig. 4).

Similarly, 16 mice aged 12 weeks were infected with *P.v.c.* and RPV, and 15 with *P.v.c.* alone. Thirteen of the former and three of the latter died. Reticulocyte levels were similar in both groups, rising from less than 1% on day 0 to 40 to 50% on day 10. No antibody could be detected at a titre of 1:10 in the doubly infected mice, which were very sick for several days before death.

Fig. 2. Patterns of infection and reticulocytosis in mice infected with both Rowson-Parr virus (RPV) and *Plasmodium berghei yoelii* (*P.b.y.*) and with *P.b.y.* alone. ○—○, RPV plus *P.b.y.*, parasitaemia; ○····○, RPV plus *P.b.y.*, reticulocytosis; ●—●, *P.b.y.*, parasitaemia; ●····●, *P.b.y.*, reticulocytosis.

DISCUSSION

Mice infected with Rowson-Parr virus succumbed to fatal infections with *Plasmodium vinckei chabaudi* and *Plasmodium berghei yoelii* whereas in virus-free animals the infections were much milder. Antibody levels in mice infected with RPV and *P.b.y.* were depressed compared with the levels in mice infected with the malaria parasite alone. In mice infected with RPV and *P.v.c.*, antibody could not be detected during the course of the infection although control animals had antibody titres of 1:640 ten days after infection; in the virus-infected mice the infection ran a virtually unchecked course until the mice died.

The effect of RPV on the immune response is also shown by a reduction in the number of plaque-forming cells to sheep erythrocytes, which was greatest when mice were infected

Fig. 3. Patterns of infection and antibody production in mice infected with both Rowson-Parr virus (RPV) and *Plasmodium berghei yoelii* (*P.b.y.*) and with *P.b.y.* alone. ○—○, RPV plus *P.b.y.*, parasitaemia; ○····○, RPV plus *P.b.y.* antibody levels; ●—●, *P.b.y.*, parasitaemia; ●····●, *P.b.y.*, antibody levels.

4 to 5 days before the sheep cells were injected. The number of mice dying from *P.b.y.* infections was also greatest when the animals received virus 4 to 5 days before the malaria parasite. In the present experiments reticulocyte levels were similar in mice infected with the malaria parasites and RPV, and in controls infected with malaria alone. Rowson-Parr virus enhances and prolongs infections of mice with *Babesia microti* which invades mature erythrocytes (Cox & Wedderburn, 1972), but the doubly infected animals did not die as did those infected with malaria and virus. Mice immunosuppressed with betamethasone and infected with *P.v.c.* (which also preferentially invades mature red cells) died with overwhelming infections (Cox, 1974) whereas those similarly treated but infected with *B. microti* all survived, though after prolonged and enhanced infections (Young & Cox, 1971); antibody levels to both parasites were depressed. In mice treated with betamethasone and infected

Fig. 4. Parasitaemia in mice infected with both Rowson-Parr virus (RPV) and *Plasmodium vinckei chabaudi* (*P.v.c.*) and with *P.v.c.* alone. ○—○, RPV plus *P.v.c.*; ●—●, *P.v.c.*

with *P.b.y.* (which requires reticulocytes for its development) antibody levels were depressed but the infections were very low because betamethasone inhibits reticulocytosis (Cox, 1974).

As we obtained similar results with both species of *Plasmodium*, and also with *B. microti*, it is unlikely that reticulocytosis was important in modifying the outcome of the infection. The exacerbating effect of RPV on *Plasmodium* infection was probably due, at least in part, to depression of antiplasmodial antibody. This depression cannot have been due entirely to binding of antibody by excess parasites since the difference in titres was marked between 7 and 12 days, when the parasitaemia in mice infected with RPV and *P.b.y.* was lower than that in the singly infected group.

Although there appears to be a relationship between antibody titre and severity of infection, there is no indication what proportion of the antibody detected by immunofluorescence is protective.

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