Enhancement and Prolongation of *Babesia microti* Infections in Mice Infected with Oncogenic Viruses

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SUMMARY

The intraerythrocytic piroplasm, *Babesia microti*, causes a self-limiting infection in Balb/c mice; parasitaemia is maximal (about 30 %) at 12 days and from 1 to 6 months after infection parasites are seldom seen in the blood. In mice concurrently infected with either of the lymphomagenic viruses Rowson Parr Virus (RPV) or ULV, a virus isolated from a urethane induced leukaemia, *B. microti* parasitaemias reached over 60 % and remained patent until the experiment was terminated at 6 months. Prolonged patent parasitaemias were higher in mice infected with ULV. Antibody levels to the piroplasm, estimated by an indirect fluorescent antibody technique, were similar in mice with and without viral infections. There was no evidence of increased lymphomagenesis in doubly infected animals.

INTRODUCTION

Infections with intraerythrocytic protozoa both affect and are affected by other agents. Most observations on these interactions have been made on infections with malaria parasites, but the general principles involved may be applicable to the other main group of intraerythrocytic protozoa, the piroplasms. The intensity of infections with *Plasmodium berghei berghei* is reduced by prior or simultaneous infection with, for example, *Eperythrozoon coccoides* (Peters, 1965, and others), West Nile Virus (Yoeli, Becker & Bernkopf, 1955) and Newcastle Disease Virus (Jahiel, Nussenzweig, Vanderberg & Vilcek, 1968; Schultz, Huang & Gordon, 1968). *Eperythrozoon coccoides* also has an antagonistic effect on infections with *Plasmodium vinckei vinckei* (Cox, 1966), *P. vinckei chabaudi* (Peters, 1967) and *Babesia rodhaini* (Peters, 1965). On the other hand, the Rowson Parr virus (RPV) which was isolated from Friend virus by end-point dilution (Rowson & Parr, 1970) and ULV, a lymphomagenic virus isolated from a urethane-induced lymphoma (Salaman, 1963; Salaman & Flocks, 1964), enhance *P. berghei yoelii* infections in Balb/c mice (Salaman & Wedderburn, 1969; Salaman, Wedderburn & Bruce-Chwatt, 1969).

Babesia microti, a common and widespread parasite of small mammals which can infect monkeys (Shortt & Blackie, 1965) and humans (Gleason *et al.* 1970) can be adapted to mice (Cox & Young, 1969). After the inoculation of infected blood, the parasitaemia reaches a peak (at which about 30 % of the red blood corpuscles are infected) 12 days later, after which the parasites disappear from the blood during the 3rd or 4th week after infection. Occasional natural recrudescences at very low levels and recrudescences after splenectomy may occur during several months after the original disappearance of parasites from the blood.

The experiments described in this paper are concerned with the effects of RPV and ULV on *Babesia microti* infections in mice.

METHODS

Mice

Balb/c mice, maintained by brother-sister mating, were fed a pellet diet, Diet 41B (W. Lillico and Sons, Betchworth, Surrey) and water *ad lib*.

Viruses

Rowson Parr Virus (RPV). Citrated plasma from infected mice was inoculated intravenously. Each dose of 0.1 ml contained 10^3 – 10^4 LD₅₀.

Urethane Leukaemia Virus (ULV). This virus is transmissible by infection or spontaneously from parent to offspring (vertically). Balb/c mice were infected neonatally and then used for breeding. This vertically transmitted line has been maintained for 18 generations without significant alteration in the incidence of lymphomas which develop after 5 to 12 months. Spontaneously infected mice were used in these experiments.

Babesia

The King's 67 strain of *Babesia microti* used in this study was originally isolated from a vole, *Microtus agrestis*, and established first in rats and later in mice. The strain was passaged weekly by intraperitoneal inoculations of infected blood in Alsever's solution (NaCl, 0.525 g; Na citrate, 1.0 g; glucose 2.33 g; distilled water, 100 ml). Mice were infected intraperitoneally with 1.25×10^6 parasitized red blood cells in 0.4 ml Alsever's solution. Infections were monitored by taking blood from a tail vein and estimating the parasitaemia as a percentage of the red blood cells infected in thin films stained with Giemsa's stain.

Immunological techniques

Antibody levels to *Babesia microti* were estimated using a fluorescent antibody technique. Thin blood films containing *B. microti* were made, dried, fixed in 0.3 N-HCl for 5 min and washed. Serial dilutions of test sera were applied for 30 min at room temperature. After staining with fluorescein labelled rabbit anti-mouse immunoglobulin for 30 min, slides were rinsed in Evans blue, mounted in glycerol and examined under ultraviolet light using a Zeiss microscope (Cox, Crandall & Turner, 1969).

Experimental procedure

Experiment 1. Four groups of six 8-week-old male Balb/c mice were treated as follows. Group 1 received *Babesia microti* on day 0, group 2 received RPV on day -5 and *B. microti* on day 0; group 3 received RPV immediately before *B. microti* on day 0 and group 4, already carrying ULV, received *B. microti* on day 0. All mice were examined at first every 2 days and then weekly for piroplasms; after 6 months they were killed and their organs examined for evidence of lymphoma.

Experiment 2. Three groups of six 8-week-old female Balb/c mice were treated as follows. Group 1 received *Babesia microti* on day 0, group 2 received *B. microti* and RPV on day 0, and group 3 received RPV alone. Two groups of ULV-infected male mice were also used. Group 4 received *B. microti* on day 0 and group 5 was used as a control. Samples of blood were taken from all these mice for immunological analysis 7 and 14 days after infection with *B. microti*.

Fig. 1. Patterns of parasitaemia in mice infected with *Babesia microti*, *B. microti* and RPV or *B. microti* and ULV during the first 5 weeks after infection. Each point is the average from six mice, plotted on a 3-cycle logarithmic scale. *B. microti* alone, —; *B. microti* and RPV on day -5,; *B. microti* and RPV on day 0, ----; *B. microti* and ULV, — — —.

RESULTS

Patterns of Babesia microti infections. The patterns of parasitaemia in Expt 1 are shown in Fig. 1 and 2.

Parasitaemia was maximal on day 12 in all groups, but the peak levels reached in the virus-infected mice were higher than those in the control group infected with *Babesia microti* alone and, while the former mice were hunched and fluffed for several days, the latter appeared healthy.

In the control group occasional parasites were seen over the next 5 months, but never more than one or two parasites in the blood film of a single mouse.

In the doubly infected mice, parasitaemia declined more slowly than in control mice, and no mouse was completely aparasitaemic during the course of the experiment. In particular, parasitaemia in the ULV-infected group persisted at a higher level than in either of the other infected groups and individual parasitaemias of up to 20 % were observed in these mice for as long as 6 months after infection (Fig. 2). There was no evidence of any cyclical pattern of recrudescence in any group.

The results obtained in the second experiment were consistent with those in the first, namely an increase in the level and duration of parasitaemia in the virus-infected mice compared with the control animals.

Antibody levels. The antibody levels in the mice in the second experiment are shown in Table 1. Titres in virus-infected animals were not significantly different from those in mice infected with *Babesia microti* alone.

Pathology. Animals infected with *Babesia microti* only, and killed at the time of peak

Fig. 2. Parasitaemia in mice infected with *Babesia microti*, *B. microti* and RPV or *B. microti* and ULV over a period of 6 months after infection. The high parasitaemias at 2 weeks (see Fig. 1) have all been reduced to 10 % for clarity. (Arithmetic scale.)

Table 1. Antibody titres in mice infected with *Babesia microti*, *B. microti* and ULV, *B. microti* and RPV, RPV or ULV

Each figure represents the reciprocal of the antibody titre determined from the pooled sera of six mice using an indirect fluorescent antibody technique.

	Days after infection with <i>Babesia microti</i>	
	7	14
<i>B. microti</i> +RPV	40	320
<i>B. microti</i> +ULV	80	320
<i>B. microti</i>	40	160
RPV	—	0
ULV	—	0

parasitaemia, 11 or 12 days after infection, had spleens weighing between 0.4 and 1.0 g. There was considerable hyperplasia of the red pulp with erythropoiesis and an increase in reticulum cells. Lymphoid follicles were also enlarged and contained active germinal centres, cells in mitosis and tingible bodies. The livers showed islands of erythropoiesis and the sinusoids were infiltrated with polymorphonuclear leukocytes and mononuclear cells.

Six months after infection, the mean spleen weight was 0.18 g. Spleens contained some pigment but the hyperplasia of the red pulp had subsided. The follicles were enlarged and active. Minimal changes remained in the liver apart from a slight hyperplasia of the Kupffer cells.

Doubly infected animals showed a mainly mononuclear cell infiltration of the liver sinusoids which was not seen at this time in mice infected with either the parasite or one of the viruses alone. They also showed a generalized hyperplasia of both the red and the white pulp of the spleen.

One of the six ULV-infected mice with *Babesia microti* became lymphomatous at 5.5 months and another showed histological evidence of neoplastic involvement when killed at 8 months of age. The 12 animals infected with RPV and *B. microti* showed no sign of overt lymphoma 6 months later.

DISCUSSION

The experiments described above show that, in mice infected with either of the oncogenic viruses RPV or ULV, the level and duration of *Babesia microti* parasitaemia is considerably greater than in mice which have not received these viruses. When *Plasmodium berghei yoelii* was combined with RPV, or with ULV (Salaman & Wedderburn, 1969; Salaman *et al.* 1969) and *P. vinckei chabaudi* with RPV (F. E. G. Cox & N. Wedderburn, unpublished) nearly all the virus-infected mice died with overwhelming parasitaemia, whereas most of the mice which had not received virus recovered. Thus these two viruses enhance infections caused by malaria parasites or piroplasms in mice.

Plasmodium berghei yoelii has a predilection for reticulocytes, and an agent which stimulates the production of these cells would be expected to enhance the infection. *Babesia microti* and *P. vinckei chabaudi*, unlike *P. berghei yoelii*, infect mature red cells preferentially, and an increase in the production of reticulocytes would be likely to depress, rather than enhance, the infection. It appears unlikely, then, that a virus-induced change in the relative numbers of reticulocytes and mature red cells could be the cause of the enhanced protozoan infections recorded in this and previous studies.

Several oncogenic viruses are known to be immuno-depressive (Salaman, 1969, 1970; Notkins, Mergenhagen & Howard, 1970). RPV sharply depresses the immune response to sheep erythrocytes over a short period early in the infection (Carter *et al.* 1970) whereas ULV exerts a moderate and gradually increasing effect during the lifetime of the infected animal (Wedderburn, 1969). In the present study the *Babesia*-specific antibody levels detected by the indirect fluorescent antibody technique were similar in the groups of mice which had received viruses and those which had not. However, protective antibody may be only a small part of the total, and this part may conceivably be specifically depressed. Young & Cox (1971) found that daily doses of betamethasone caused enhancement of *Babesia microti* infections comparable with those recorded in this study without any alteration in antibody levels as detected by the indirect fluorescent antibody technique.

Interferon and interferon inducers have inhibitory effects on infections with *Plasmodium berghei berghei* (Jahiel *et al.* 1968; Schultz, Huang & Gordon, 1968). Friend virus, from which RPV was isolated, inhibits the production of interferon by Sendai virus (Wheelock,

1966). If interferon affects piroplasmiasis in the same way as it does plasmodial infections, the enhancement of parasitaemia in virally infected animals might be due, at least in part, to this kind of interaction.

The incidence of *Babesia microti* in small mammal populations, as estimated by an examination of blood films, has been shown by Young (1970) to be higher than would be expected if the period of patency in nature is only 3 to 4 weeks, as it is in the laboratory. RPV and ULV were isolated from laboratory animals but a number of oncogenic viruses, some of similar type, have been isolated from wild rodents (Rowe, Huebner & Hartley, 1960; Pope, 1961, 1963; Gardner *et al.* 1971), as have some non-oncogenic viruses which are immuno-depressive for short periods (Mims & Wainwright, 1968; Osborn, Blazkovek & Walker, 1968). Prolonged parasitaemias are usually attributed to antigenic variation in the parasite, but the present observations suggest that they could also result from concurrent viral infections which have not been detected because they have not been looked for. It is also possible that the duration of laboratory infections with *B. microti* may sometimes be altered by unrecognized latent virus infections. This might account for the continuous high parasitaemias recorded by Irvin & Brocklesby (1969) with the same strain of *B. microti* as used in the present study, but using BSVS mice.

The possible effect of *Babesia microti* on the development of lymphoma in mice infected with either RPV or ULV has not been investigated fully in the present work. The effects of two other oncogenic viruses, murine sarcoma virus (Harvey) and Moloney virus, are enhanced by concurrent infection with *Plasmodium berghei yoelii* (Salaman *et al.* 1969; Wedderburn, 1970). Almost all Balb/c mice infected with RPV as young adults develop lymphoma between 6 and 12 months later (Carter *et al.* 1970) and 50 to 80 % of ULV-infected mice develop lymphoma between 5 and 12 months.

There was no evidence of enhanced lymphomagenesis in this study. However, the design of the experiments and the numbers of animals used were not such that this possibility can be excluded.

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