

## Isolation of a Murine Hepatitis Virus from Swiss Mice Treated with Antilymphocyte Serum

By

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### Summary

In an attempt to transmit feline malignant lymphoma to mice, a murine hepatitis virus (MHV) was accidentally recovered from conventionally reared Swiss mice receiving prolonged treatment with antilymphocyte serum. In these mice, the virus did not require concomitant infection with *Eperythrozoon coccoides* to produce disease. Tests for antibodies against a variety of viruses were performed on serum from control colony mice and mice that recovered from experimental infection as well as on serum pooled from vaccinated and non-vaccinated mice challenged with the recovered agent. Antibodies to the MHV complement fixing (CF) antigen(s) were demonstrated in only the last mentioned serum. Mice harbouring a hepatitis virus may thus be tolerant to their CF antigen(s) in a situation analogous to oncornavirus infection of their natural hosts. The liver pathology was that of a confluent focal-type necrosis resembling that produced by certain other MHV strains. We have labelled this newly isolated virus MHV (Swiss-Cape Town), abbreviated to MHV (S-CT).

### 1. Introduction

Immunodepression with antilymphocyte serum (ALS) has been documented as causing latent viruses to manifest themselves in animals (21). In 1971, ALLISON<sup>1</sup> reported that during work on oncogenesis some ALS-treated mice developed hepatitis. He considered that this was probably the result of potentiation of the pathogenic effects of a mild strain of MHV by the immunosuppression.

The purpose of this communication is to record the accidental recovery of a murine hepatitis virus (MHV) from mice of conventionally reared stock that received prolonged treatment with ALS in an attempt to transmit feline malignant

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lymphoma to them. In addition, we have serially transmitted the disease and documented the serological findings.

## 2. Materials and Methods

### 2.1. Mice

Swiss albino mice were obtained from the conventionally reared colony of the University of Cape Town Medical School.

### 2.2. Antilymphocyte Serum

Rabbit anti-mouse-lymphocyte serum was prepared according to the two-pulse procedure of LEVEY and MEDAWAR (13). It was not assayed for immunodepressive potency.

### 2.3. Bacteriology

Tissues and ascitic fluid were examined microscopically and, using standard procedures, were cultured aerobically and anaerobically for bacteria and mycoplasma.

### 2.4. Histopathology

Tissues for light microscopy were fixed in formaldehyde solution (10 per cent formalin) and sections were stained with haematoxylin-eosin. Blood smears and liver imprint preparations were stained with either Giemsa or Leishman stain.

### 2.5. Serology

Serum samples were sent to Microbiologic Associates, Inc., Bethesda, U.S.A. for antibody testing to 11 different viruses including MHV, as recommended by the firm for virus screening of experimental mice.

### 2.6. Vaccine

Fresh mouse liver tissue infected with the isolated agent in its eighth murine passage was ground in a Ten-Broek grinder and suspended 1:5 (w/v) in PBS containing 200 IU of penicillin and 200  $\mu$ g of streptomycin per ml. The suspension was lightly centrifuged and formalin was added to the supernatant fluid to give a 0.1 per cent final concentration. This material was held at 4° C for 2 weeks prior to use as vaccine.

## 3. Results

### 3.1. Isolation and Experimental Transmission

In the attempt to transmit malignant feline lymphoma to mice, a small fresh piece of a lymphomatous spleen from a cat was implanted subcutaneously in each of a group of 3-week old mice. Immediately thereafter each mouse was injected intraperitoneally with 0.5 ml of ALS and this was repeated on the second and fourth days. Thereafter the dose of ALS was reduced to 0.2 ml administered via the same route every 2 to 4 days. Thirty five days after commencing the experiment a moribund mouse was observed and was killed.

At autopsy it was found to be emaciated. The liver was enlarged and both the capsular and cut surface showed the presence of multiple, yellowish necrotic foci. These varied in size from less than 1 mm to about 5 mm in diameter. There was some straw-coloured ascitic fluid but no other macroscopic features of note in any of the organs. Bacteriological examination revealed some coliform organisms in the peritoneal fluid but was otherwise completely negative.

The disease was first transmitted experimentally to non-ALS treated mice using a 1:5 (w/v) pooled organ suspension of liver, kidney, and spleen in PBS containing 200 IU of penicillin and 200  $\mu$ g of streptomycin per ml. This suspension was held at 4° C for 2 days prior to injection of 0.5 ml amounts intraperitoneally in each mouse. The disease has been serially transmitted 9 more times in mice of varying ages by intraperitoneal injections of either infected liver or liver plus spleen suspensions in PBS with the above antibiotics. Incidentally, transmission of the disease was also achieved using either serum, blood clot suspension, or spleen suspension from infected mice. Oxytetracycline treatment of a liver suspension from diseased mice did not destroy infectivity for other mice (see below). Furthermore, the infectious agent passed through a 0.45  $\mu$  pore diameter Swinnex 13 millipore filter (Millipore Corp., Bedford, Mass., U.S.A.).

Observations made during the routine transmission of the disease indicated that younger mice were more susceptible than older ones. In addition, certain litters were more resistant than others, reflecting genetic differences in the randomly bred stock as well as possible differences in immune status, *e.g.*, passively acquired immunity from the mothers. When large doses of infectious material were injected intraperitoneally into mice varying in age from 1 to 14 days, clinical disease was first observed 3 to 6 days later.

### 3.2. Bacteriological Findings

On one occasion during passage of the agent, *Staphylococcus* and *Pseudomonas aeruginosa* were cultured from the liver and *Clostridium bifermentans* from the spleen of a mouse with hepatitis. However, on the 6 other occasions when bacteriological examination of either single or pooled livers from mice suffering from the liver disease were conducted, no bacteria were isolated. No mycoplasma organisms were isolated from diseased livers.

### 3.3. Examinations for *Eperythrozoon coccoides*

Blood smears and liver imprint smears from control mice from the breeding colony, mice injected 5 days previously with heart blood from colony mice, or mice suffering from severe, experimentally induced hepatitis failed to reveal evidence of *E. coccoides* (17, 18). The course of the disease was not altered by addition of high concentrations of oxytetracycline to the infectious material.

### 3.4. Serological Findings

Pooled serum samples were sent to Microbiologic Associates, Inc. The first, from 50 killed control mice of either sex from the colony, contained haemagglutination-inhibition (HI) antibodies in a titre of 1:40 to both Theiler's encephalomyelitis virus (GD VII) and minute virus of mice (MVM) and was negative for antibodies against the 9 other viruses. The second serum pool, from 12 female weanling mice bled about 2 weeks after recovery from experimental infection with sublethal concentrations of infectious material, revealed no antibodies to the 11 test viruses. The third combined serum pool was from a batch of mice initially vaccinated intraperitoneally with either 0.1, 0.2 or 0.5 ml of vaccine at about 1 week of age and subsequently challenged 22 and 35 days later with infectious material via the same route, as well as from a batch of 9 females that had survived

2 injections with the same infectious material without having first been vaccinated. This pool had an HI antibody titre of 1:20 to pneumonia virus of mice (PVM) and a MHV complement fixing antibody titre of 1:40 but was negative for antibodies against the other viruses.

### 3.5. Pathology

Mice suffering from the experimentally induced lesion were killed when obviously ill. The macroscopical features at autopsy were identical to those described in the mouse from which the isolation was made, namely, a variable amount of ascitic fluid and pale foci of necrosis in the liver (Fig. 1). Microscopical examination

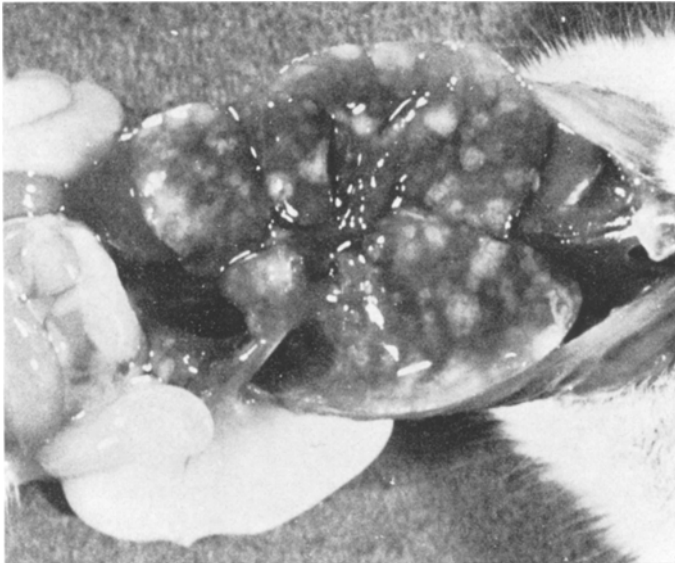


Fig. 1. Mouse liver at autopsy showing multiple, pale, necrotic foci

of the livers showed irregular areas of coagulative necrosis usually involving part of a lobule but often extending into adjacent lobules (confluent necrosis) (Fig. 2). The necrotic areas were randomly distributed within the lobule, in some lobules abutting on central veins and in others being periportal in location. The necrosis was associated with a patchy mononuclear cell infiltrate in which lymphocytes and macrophages predominated. Moderate numbers of mitotic figures were noted within parenchymal cells indicating an attempt at regeneration (Fig. 3). No viral inclusion bodies were seen. Routine sections of other organs showed no features of note.

### 4. Discussion

In an attempt to transmit feline malignant lymphoma to mice receiving treatment with ALS, one of the animals unexpectedly developed a fulminating hepatitis. The disease was readily transmissible in non-ALS treated mice by means of serum or organ suspensions from infected animals and to date has been passaged serially 10 times.

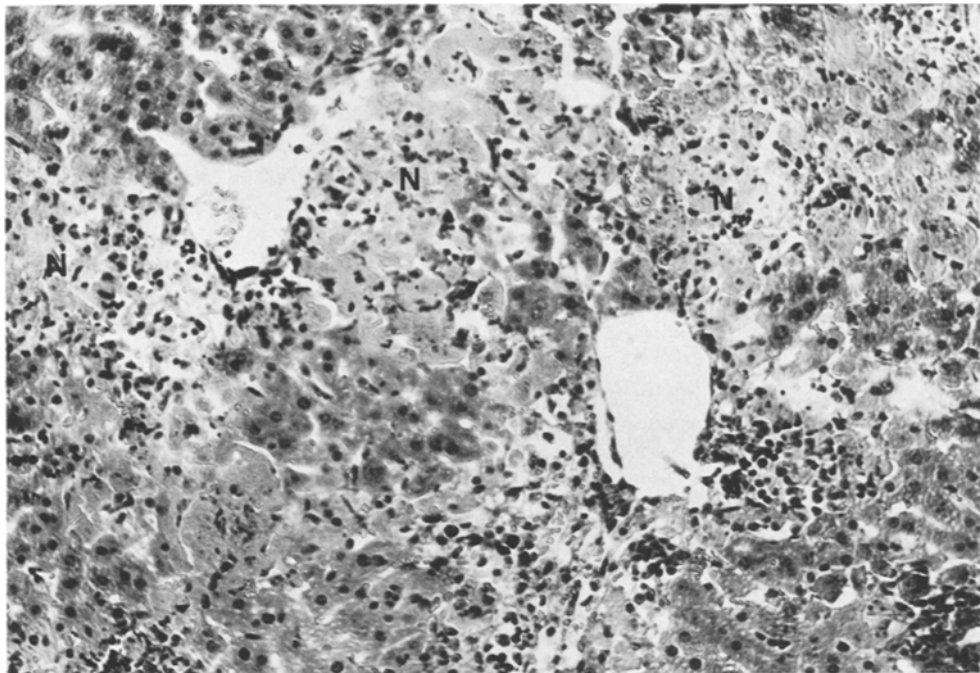


Fig. 2. Photomicrograph of mouse liver showing areas of focal necrosis (N) which are becoming confluent. A patchy, chronic inflammatory cell infiltrate is also seen. Haematoxylin-eosin,  $\times 250$

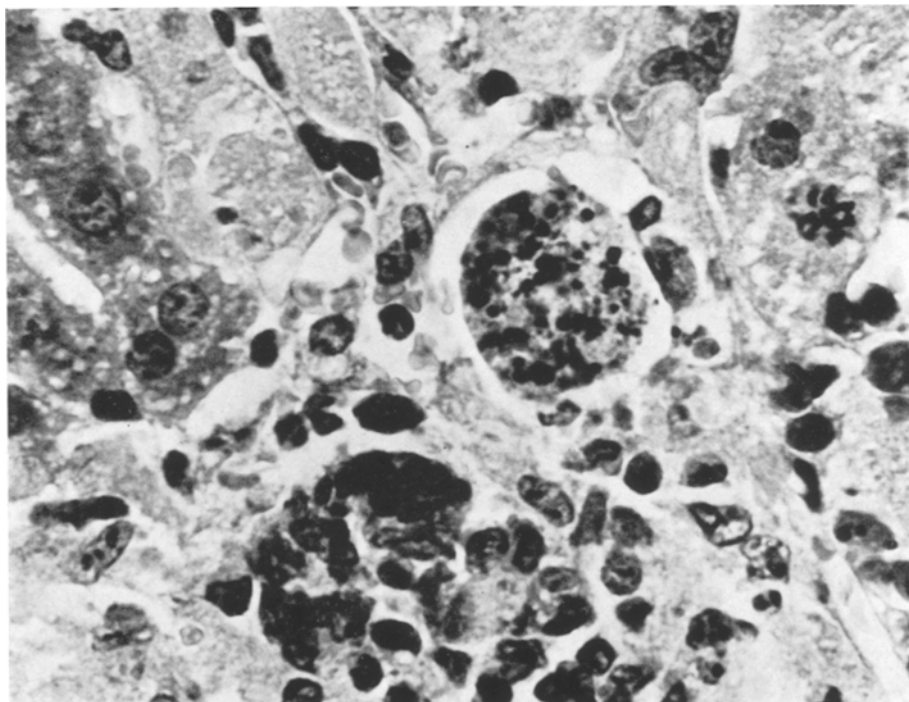


Fig. 3. High power photomicrograph of mouse liver showing many necrotic liver cells. Karyorrhectic nuclear debris is seen in the centre of the picture, a liver cell in mitosis in the right upper corner and a round cell infiltrate towards the bottom. Haematoxylin-eosin,  $\times 800$

We believe that the causative agent is a virus. It passed through a 0.45  $\mu$  millipore filter and was resistant to several antibodies, including tetracycline. Murine hepatitis virus CF antibodies were detected in serum pooled from non-vaccinated and vaccinated mice that were challenged with the isolated agent. The pattern of confluent zonal necrosis associated with a round cell infiltration of the liver resembled that produced by other MHV strains (16), and was quite unlike the pyogenic lesions which would be expected in a bacterial infection. Cultures of diseased livers for bacteria including mycoplasmas were negative on several occasions. The detection of MHV CF antibodies as well as the localization of the disease to the liver would suggest the virus is a MHV strain. The other 3 viruses to which antibodies were demonstrated are not known to cause murine hepatitis. We designated this strain of MHV as MHV (Swiss-Cape Town), abbreviated to MHV (S-CT).

Some strains of MHV require co-infection of their hosts with *E. coccoides* in order to express their pathogenicity (7, 9, 14, 16). No evidence was found of *E. coccoides* in the University of Cape Town colony. In addition, *E. coccoides*, is known to be highly susceptible to tetracyclines (8, 16), yet in the present study the lesion was still produced following treatment of infectious material with oxytetracycline. Thus MHV (S-CT), at least in Swiss mice, is capable of producing hepatitis independently of *E. coccoides*.

The variable results obtained in the serologic screening tests for viruses conducted on our serum pools indicate the shortcomings of such tests for determining the virus status of stock laboratory mice. Serial passage of organ material combined with frequent serologic testing would be more reliable.

The failure to detect CF antibodies to MHV in the serum samples of either the control mice or the mice that had recovered from hepatitis could be a result of inadequate sensitivity of the test. Alternatively, the mice may be tolerant to the CF antigen(s) in a situation analogous to the tolerance displayed by animals to homologous group specific oncornavirus antigen (12). It may be relevant in this connection that coronaviruses in some ways resemble oncornaviruses (2). If the latter hypothesis is correct, the presence of CF antibodies in the serum pool from the challenged non-vaccinated and vaccinated mice could be attributed to abrogation of tolerance in the vaccinated mice by the formalin modified antigen. Termination of tolerance by related or modified antigen is a well recognized phenomenon (3, 19, 20).

The appearance of the disease in the mouse from which the agent was recovered could have resulted from viral activation secondary to stress caused by the frequent treatment with ALS. Alternatively, viral activation may have resulted specifically from depression of immunity by ALS. Even if mice latently infected with MHV are tolerant to its CF antigen(s), it seems likely that mechanisms such as formation of neutralizing antibodies against the virus, cell mediated immunity, phagocytosis and interferon production exist that maintain the host-parasite equilibrium and prevent the development of overt disease. Recovery of latently infected mice following moderate "superinfection" may result from enhancement of the above-mentioned mechanisms.

Mice latently infected with MHV thus appear to be in a state of delicate host-parasite equilibrium dependent on a variety of factors. A similar complicated

interplay of factors is probably also responsible for the sporadic occurrence and unpredictability of the course of hepatitis in other lower animals and man. It also raises the possibility of development of virus hepatitis in man receiving ALS or antilymphocyte globulin (ALG). In 1968 EVANS *et al.* reported liver damage in 5 patients following immunosuppressive therapy (4). In 2 of the patients the treatment included the administration of ALG. He considered hepatotoxic drugs, virus hepatitis and cytomegalovirus as possible causative factors. More recently, cases have been documented associating immunosuppressive therapy with hepatitis, and the presence of hepatitis B antigen in human patients (5, 6, 10, 11).

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