HISTOPATHOLOGICAL CHARACTERIZATION OF THE NATURALLY OCCURRING HEPATOTROPIC VIRUS INFECTIONS OF NUDE MICE

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SUMMARY

In the last ten years the mutant athymic nude mouse has been used by many researchers as a model for studying pathological conditions in an immunodeficient host. A major problem with such mice is their susceptibility to viral infections indigenous to murine stocks, such as the hepatotropic mouse coronaviruses. In an effort to define the hepatotropic virus diseases indigenous to nude mice this paper details the pathogenesis and associated comparative histopathology of three common strains of mouse hepatitis virus, reovirus 3 and murine cytomegalovirus in the nude mouse.

KEY WORDS—Nude mice, hepatitis, viruses, hepatotropic viruses.

INTRODUCTION

The effect of naturally occurring virus diseases in nude mice has been studied by several authors. Respiratory viral infections caused by sendai virus have been described by Ward et al.1 and Ueda et al.2 Carthew and Sparrow3 compared the pathogenesis of sendai virus infection with that of pneumonia virus of mice in the germ free nude mouse. In terms of hepatotropic virus infections, natural outbreaks of mouse hepatitis virus (MHV) infection has been described by Sebesteny and Hill,4 Tamura et al.,5 and Ward et al.6 Carthew7 compared the histopathology and pathogenesis of mouse hepatitis type 1 virus induced experimentally in the nude mouse, with that of field cases observed in the United Kingdom and concluded that they were similar. They were also indistinguishable from the natural infections described in Japan.5

As no other experimental studies in nude mice have been recorded for other strains of mouse hepatitis virus (Types S, A59, 3) it would seem that the differentiation of these infections would have to rely upon neutralization typing of virus isolations with antiserum produced against these strains, which is not a common procedure, due to the extensive serological cross-reactions between the strains of MHV. However, it is relatively simple to prepare histological sections of livers from nude mice exhibiting hepatic lesions, and it would be advantageous if the different strains of hepatitis virus could be distinguished by a combination of information on the virulence of a strain of virus and the histopathological appearance of the liver lesions. To determine whether this is possible, the pathogenesis of three strains of mouse hepatitis virus (1, A59 and S) was compared in nude mice. The histopathological appearance of the lesions relative to two other common murine hepatotropic viruses, as yet undocumented in nude mice, is also described.
MATERIALS AND METHODS

Viruses

Mouse hepatitis viruses (MHV) types 1, A59, S; and Reovirus Type 3 (Abney strain) were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A. Murine cytomegalovirus was the kind gift of Professor C. A. Mims, Guy's Hospital Medical School, London Bridge, London, England. The three strains of mouse hepatitis were all propagated and titrated in NCTC 1469 cells. Reovirus 3 was propagated and titrated in BHK 21 cells. Murine cytomegalovirus (MCMV) was prepared in nude mice by intraperitoneal injection, and harvest of the salivary glands 2 weeks later. Resultant homogenized salivary gland virus was titrated in primary mouse embryo cells as described by Henson and Pinkerton. 8

Animals and infection procedures

Outbred homozygous nu/nu nude mice 6–8 weeks old were used for all experimental infections. All virus suspensions were administered intraperitoneally and the mice were maintained in filter boxes for the duration of the experiments. Mice were dosed with one of the following viral suspensions: (1) MHV A59 0.2 ml of 2×10^2 50 per cent tissue culture infective doses or a hundred-fold dilution of this suspension. (2) 0.2 ml of 10^3 or 10^2 50 per cent tissue culture infective doses of MHV S and 1. (3) 0.2 ml of 10^5 or 10^4 50 per cent tissue culture infective doses of Reovirus 3. (4) 2×10^3 plaque forming units per mouse of murine cytomegalovirus. Duplicate samples of livers from mice infected with MHV A59 were taken daily after infection and for the other viruses duplicate daily samples were taken for the first three weeks, then at weekly intervals until the end of the experimental period.

Histology

Liver samples were fixed in Bouin’s fixative overnight then wax embedded. 5 μm sections were cut and stained with haematoxylin (Erlich’s) and eosin.

RESULTS

Pathogenesis of different murine hepatitis virus strains

The most lethal of the three strains of murine hepatitis virus was strain A59. Nude mice infected with this virus usually died in 4 days and even when the dose was reduced one hundred-fold the mice only survived to 6–7 days post infection. The histopathology produced by this strain of virus was quite straightforward, an incipient multifocal necrosis observable 48 h after infection, which progressed very rapidly so that the focal lesions coalesced to destroy nearly all of the liver parenchyma in such a short span of time that there was no cellular response observed. Parenchymal cells adjacent to hepatic veins appeared to survive the longest (Fig. 1).

MHV Type S produced a more chronic type of infection of reduced virulence. It made no difference in overall survival time whether the nude mice were given 10^3 or 10^2 50 per cent tissue culture infectious doses of this strain. The maximum time that any of these mice survived was 3 weeks. The histopathology produced by this strain was much more typical of a chronic infection

| Virus  | Survival time (days) | Necrotic parenchymal lesions | Giant cell formation | Parenchymal fibrosis | Fibrosis and bile duct hyperplasia |
|--------|---------------------|----------------------------|---------------------|---------------------|----------------------------------|
| MHV A59 | 4–7                | +++                        | –                   | –                   | –                                 |
| MHV S   | 19–21              | +                          | +++                 | +                   | –                                 |
| MHV 1   | 60–90              | +                          | ++                  | +++                 | –                                 |
| Reo 3   | >180               | +                          | –                   | –                   | +++                               |
| MCMV    | 20–24              | +                          | –                   | +                   | –                                 |

*Scale of severity of condition: – negative; + small; ++ moderate; +++ pronounced.
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Fig. 1—Focal necrotic liver lesions coalescing, 4 days post infection with MHV A59. H & E, ×160

Fig. 2—Focal necrotic liver lesion in Type S infection after 7 days. Note infiltration and characteristic giant cell formation at periphery of lesion. H & E, ×160
Fig. 3—Fibrotic liver lesions 10 days after infection with MHV S. Some giant cells show pyknotic nuclei. H & E, ×160

Fig. 4—Degenerating syncytia formed during infection with MHV 1. H & E, ×240
Fig. 5—Fibrotic lesion formed 25 days after infection with MHV 1. Note nests of polymorphonuclear leucocytes adjacent to the portal tract. H & E, ×160

Fig. 6—Inflammation around portal tract of the liver during reovirus 3 infection with an adjacent ‘holey’ focal lesion, 10 days post infection. H & E, ×160
in the liver. Focal necrotic lesions were observed within 3 days of infection but unlike type A59 they did not expand and coalesce. They enlarged slowly and characteristic multinucleate giant cells (syncytiata) were associated with the lesions (Fig. 2). Because the lesions progressed slowly there was an infiltrative response of polymorphonuclear leucocytes and lymphocytes. After 10 days fibrosis was present associated with necrosis and many giant cells (some with pyknotic nuclei) (Fig. 3).

Although the histological features of the livers in the case of Type 1 infection were very similar there were some important differences. Again \(10^3\) or \(10^2\) 50 per cent tissue culture infective doses resulted in the same pattern of deaths over a period of time; however, in the case of Type 1 hepatitis, it was common for mice to survive in some cases for periods of 2–3 months. While multinucleate giant cell formation was also a feature of infection with this strain the number of syncytia was markedly reduced (Fig. 4) and there was also a tendency for nests of polymorphonuclear leucocytes to appear adjacent to portal tracts or in the fibrotic parts of the parenchyma (Fig. 5) 3 weeks or more after infection.

It would seem therefore that Type 1 mouse hepatitis is a more chronic infection than type S and on this basis can be differentiated from the number of syncytia found and the characteristic polymorphonuclear response in the period when mice infected with type S would have died.

**Pathogenesis of reovirus 3 infection**

In contrast to the hepatitis strains examined, reovirus 3 produces a characteristic histopathology. The dose of virus given did not appear to affect the outcome of the disease as in this case it was benign. Mice infected with the virus were kept for up to 6 months with no deaths. The pattern of reovirus infection was that of a chronic biliary tract infection with inflammation of the portal tracts of the liver and also a focal parenchymal necrosis. The necrotic foci were quite distinctive; they were sharply delineated with a small margin of leucocytic cells around their edge, but not a great deal of fibrotic replacement of the parenchymal cells destroyed, giving the liver sections a 'holey' appearance (Fig. 6). The fibrotic response occurring in this infection appeared more localized to the portal tracts with biliary proliferation a marked factor of the chronic infection. A lobular fibrosis developed as the fibrotic areas around portal tracts eventually connected (Fig. 7). The focal necrotic

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**Fig. 7**— Extensive fibrosis between portal tracts after 3 months' chronic reovirus 3 infection. H & E. ×160
lesions were visible within 7–10 days after infection whereas the biliary fibrosis or bile duct hyperplasia was more marked 10–13 weeks after infection and was especially prominent in the posterior liver lobes.

Pathogenesis of chronic cytomegalovirus infection

Cytomegalovirus infection in the livers of nude mice followed a very distinctive course. Mice infected with various doses of cytomegalovirus survived for a maximum of 3–4 weeks. Small focal necrotic liver lesions were seen commencing 4 days after dosing and persisted throughout the period of infection. The lesions had an attendant lymphotic infiltration and easily visualized intranuclear inclusion bodies evident in infected parenchymal cells.

DISCUSSION

The recognition and differentiation of the different strains of mouse hepatitis virus has always been a problem due to the substantial serological cross-reactions that can occur when typing different strains.9 The fact that different strains can be distinguished pathologically is perhaps more suited to some investigators, as it does not require the preparation of numerous different serological reagents and the use of a tissue culture cell line which is not commonly available (NCTC 1469 cells). Broadly speaking the different strains of hepatitis virus can be divided into two pathological types. Types 3, A59 and JHM are known to be highly virulent10,11 and therefore do not constitute a difficulty in histopathological diagnosis, once hepatitis virus has been identified as the cause of the lesions.12 Types 1 and S are much less pathogenic and can be identified from their chronic pathology11 and distinguished from one another on the basis of the differing relative numbers of syncytia and polymorphonuclear cells present.

The histopathology produced by infection with reovirus 3 is so distinctive that it is a relatively simple matter to distinguish the unusual focal necrotic lesions and the portal tract fibrosis and bile duct hyperplasia characteristic of the infection in the nude mouse. Finally, cytomegalovirus infection cannot be confused with any of the above, as the distinctive intranuclear inclusion bodies, which are the hallmark of herpetoviridae infections in general, are always present in parenchymal cells of the liver.13

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