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PATHOLOGICAL LESIONS IN MICE INFECTED WITH THOGOTO VIRUS, A TICK-BORNE ORTHOMYXOVIRUS

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Summary. — The RNA structure of tick-borne Thogoto virus was found very closely related to that of the *Orthomyxovividae* family. Pathological lesions in the lungs, liver and intestine of white mice inoculated with Thogoto virus resemble to those described for influenza virus, however, an important differential characteristic is the high hepatotropism of Thogoto virus. Thogoto virus infection in the liver of mice seems suitable for the study of virus-induced necrotic lesions.

Key words: Thogoto virus; tick-borne orthomyxovirus; hepatotropic tick-borne virus; arbovirus pathogeneis

Introduction

Thogoto virus is a tick-borne virus isolated over a wide geographic area, including Africa, Asia and Europe, probably because migratory birds are involved in transporting and disseminating the infected ticks. Analysis of the Thogoto viral genome has demonstrated seven size classes of single-stranded (ss) RNA, identifying this virus with the *Orthomyxoviridae* family of viruses (Clerx *et al.*, 1983). Structural studies of the tick-borne Dhori virus also isolated in Portugal (Filipe and Casals, 1979), have shown that this virus shares the common structural characteristics of Thogoto virus and the *Orthomyxoviridae* (Clerx *et al.*, 1983).

In experimental studies on albino mice infected with a strain of Thogoto virus isolated in Portugal, we found microscopic pathologic lesions that were quite distinct from those usually seen in mice inoculated with arboviruses. The most striking microscopic lesions involved the liver; intestinal haemorrhage was also observed in a large number of the animals. The pathological features resembled those described for influenza, the prototype virus of the *Orthomyxoviridae*.

Materials and Methods

Thogoto virus, strain PO Ti 503 isolated from *Rhipicephalus sanguineus* collected in Portugal in 1978 (Filipe and Calisher, 1984), had been passed 10 times in suckling mice.

Outbred albino mice (NIH general purpose strain), 2 to 4-day-old, from the specific pathogen-free colony of the Centers for Disease Control, Fort Collins, Colorado, were inoculated by the intraperitoneal (i.p.) route with 5000 Vero cell plaque-forming units (PFU). After death, 96 hr post-inoculation (p.i.) the organs were removed, weighed, and stored at −70 °C until tested. Ten percent (w/v) suspensions of tissues, clarified by centrifugation were titrated by plaque assay in Vero cell cultures grown in 6-well plastic trays as described elsewhere (Hunt and Calisher, 1979). Tissues from mice sacrified at approximately daily intervals were fixed in 10 % buffered formalin, embedded in paraffin, stained with haematoxylin-eosin, and examined by light microscopy.

Results

Mice died between 3 and 4 days p.i. In moribund animals at 96 hr p.i. high virus titres were present in all organs tested.

In mice examined at daily intervals p.i., the most remarkable histopathological finding was hepatic necrosis. At 48 hr, small necrotic foci were present, usually involving no more than 2 or 3 cells. At 72 and 96 hr, livers contained larger necrotic foci (Fig. 1). In one case microvacuolar degeneration of large numbers of hepatocytes was observed, presumably preceding the onset of necrosis. In dead mice the hepatic lesions were characterized by a diffuse necrosis, with only a few normal cells left among the cellular debris(Fig. 2). Portal congestion was seen even at earlier stages p.i. In some cases haemorrhage in the wall of gall bladder was observed with no evidence or rupture of haemorrhage into the lumen.

Pulmonary lesions were found as early as at 24 hr p.i., consisting of interstitial pneumonia with infiltration with mononuclear cells in the alveolar septa (Fig. 3). A few cases of intraalveolar haemorrhage were also seen. In the central nervous system, lesions consisted mainly of congestion and haemorrhage of the meninges, occasionally associated with inflammatory infiltrates. In some cases, significant haemorrhage into the fourth brain ventricle was found.

The intestine showed no lesions in the earlier stages of infection. By 96 hr p.i., severe congestion of the mucosa and submucosa of the small and large intestines was seen, in most cases associated with haemorrhage into the intestinal lumen. Haemorrhage was probably due to microruptures of the congested vessels, since no necrotic lesions were seen in the epithelium.

No lesions were observed in the kidney, heart, or spleen. However the mesenteric lymph nodes showed a clear enlargement and, in a few cases, necrotic changes of the reticular cells.

Discussion

Focal and diffuse necrosis of hepatic cells, similar to our own findings, were described by Albanese *et al.* (1973), in adult mice infected with Thogoto virus. Mononuclear cell infiltrates in affected livers of adult mice described

by these authors were obscured in our study, due to the extramedullary haematopoiesis in the parenchyma livers of the infant mice.

In mice infected with influenza A (strains PRS, WS, Melbourne, F-12, F-99), influenza B (Lee and ES strains), and the S-15 strain of swine influenza, necrotic lesions in the liver were evident when massive doses of virus were injected i.p. or intravenously (Henle and Henle, 1946; Mims, 1960*a*, *b*). There was no evidence for replication of this virus in the liver (Mims, 1960*b*), in contrast to our findings with Thogoto virus. Furthermore, it was not possible to transfer influenza virus by liver cell lysates, in contrast to what can be achieved with Thogoto virus. Haller (1975) selected an hepatotropic variant of avian influenza A/Turkey/England 63 (H1N3) virus after 13 in vivo passages. With this virus strain, caused liver damage and death between 54 to 62 hr p.i. No pathologic study of any other organ was made.

The pulmonary lesions described in influenza infection are very similar to those found in Thogoto virus infected mice. There was an early infiltration of mononuclear cells in the alveolar septa, corresponding to the lesions found in interstitial pneumonia.

Unadapted influenza virus strains do not usually produce overt illness in mice inoculated by the parenteral route. Massive doses of viruses, as well as multiple virus passages are necessary to obtain a pathogenic strain. Influenza virus predominantly affects the respiratory tract and death is caused by pneumonia. Titres of influenza virus are the highest in the nasal mucosa or lung (Sweet and Smith, 1980), although virus can be also recovered from other organs.

It is interesting to note that some authors found haemorrhagic enteritis in mice inoculated intravenously with influenza virus (Mims, 1960*b*). We also found haemorrhagic enteritis in mice infected with Thogoto virus; although it was not present in all of the animals, it was far more frequent than reported in influenza virus models. The pathogenesis of the enteritis remains obscure, but it might reflect capillary wall fragility during the viraemic phase. Highest viral titres were found in the intestine, which must correspond to local virus replication.

In summary, Thogoto virus is an orthomyxovirus adapted to transmission by ixodid ticks, but retaining certain biological characteristics of influenza virus. Hepatotropism and liver cytopathology associated with viral replication in the liver appear to distinguish the pathogenesis of Thogoto virus from influenza virus being an interesting model for the study of virus--induced liver pathology.

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Explanation of Micrographs (Plates XXXVIII–XXXIX):

Fig. 1. Multiple necrotic foci in the liver (72 hr p.i.) (HE × 200). — above.

Fig. 2. Diffuse hepatic necrosis found in most infected animals, from 96 hr p.i. until death. A few intact hepatocytes can be seen. (HE × 200) — below.

Fig. 3. Interstitial pneumonia. Marked infiltration with mononuclear cells in the alveolar septa. (HE × 70).