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Hemorrhagic Disease in Rodents Infected with Virus Associated with Thai Hemorrhagic Fever

Abstract. KLA 16 virus, recovered from a child with Thailand hemorrhagic fever, produces infant mouse, rat, and hamster disease that is characterized by spontaneous bleeding at multiple sites, notably in the gastrointestinal tract, and by marked abnormalities in hemostatic mechanisms. This virus differs in this respect from Chikungunya virus to which it is immunologically similar and from other Thai hemorrhagic fever viruses.

Human virus infections, characterized by spontaneous hemorrhage, are recorded from widely separated areas of Asia, Europe, and South America. Immunologically distinct agents have been implicated in the etiology of these hemorrhagic fevers, but thus far only the viruses of Kyasanur Forest Disease and Omsk, Philippine, Argentine, and Thailand hemorrhagic fevers have been propagated in laboratory hosts (*1*, *2*). These agents multiply in albino mice and produce fatal encephalitis with minimal apparent involvement of other major viscera. Recently a virus was recovered from the blood of a Thai child who contracted Thailand hemorrhagic fever in Bangkok in May 1960. This agent, KLA 16, was identified as an arthropod-borne virus of Casal's group A, closely related to Chikungunya virus and immunologically identical to TH 35 and BaH 306 viruses recovered from Thai patients by Hammon and Kitaoka, respectively (*2*, *3*). KLA 16 virus differs from these, however, in its capacity to produce hemorrhagic disease in rodents. This report describes the hemorrhage-producing characteristics of KLA 16 virus and the factors thus far known to influence production of the hemorrhagic diathesis in experimental animals.

From the patient's blood obtained on the 2nd day of disease, virus was recovered simultaneously in mice 1 to 2 days old and in trypsinized explants of hamster kidney and rhesus kidney cells. These three isolates were transmissible interchangeably in each of the three host systems and were immunologically identical. Intracerebral passage of infected brain or cell cultures resulted in subcutaneous and marked intraluminal intestinal hemorrhages in approximately 20 percent of inoculated mice. The bleeding tendency has been observed in mice inoculated with virus in as high as 12 mouse and 7 hamster kidney cell passages, with virus obtained after three terminal dilution passages in both suckling mice and hamster kidney cells.

Several factors influenced the occurrence of discernible hemorrhage after inoculation of mice (*4*). After infection with 10 to 100 intracerebral LD_{50}, several age-dependent patterns of disease were observed. Hemorrhage was seen only in mice infected before the 7th day of life. No hemorrhage, and only sporadic deaths were observed between 7 and 21 days of age, and older mice were resistant to lethal infection. The frequency and extent of hemorrhage was greatest in mice 24 to 48 hours old at inoculation. The intracerebral route of infection was more sensitive than subcutaneous or intraperitoneal inoculation for both the production of hemorrhagic disease and for quantitation of infectivity. Hemorrhage rarely followed subcutaneous or intraperitoneal inoculation of any amount of virus, and these routes were 1/100 as sensitive for detecting viable virus as intracerebral inoculation. By passing only virus from brains of hemorrhagic mice three times, it was possible to increase the frequency of overt bleeding from 20 to 90 percent. Thus intracerebral inoculation of 10 to 100 LD_{50} of this final virus seed into mice 24 to 48 hours old was found to be optimal for producing hemorrhagic disease.

Under these circumstances, normal activity of mice decreased about 72 hours after inoculation. All or portions of the small intestine were salmon pink in color, and when viewed under ×20 magnification, marked dilatation and congestion of blood vessels in the wall was seen. Within a few hours, color changed from pink to gray, and as further hemorrhage occurred into the lumen, segments or large portions of the bowel turned black (Fig. 1). Black bowel contents were strongly benzidine- and guaiac-positive; blood in the intestine was always partially digested. Subcutaneous, intra-articular, and bladder wall hemorrhages occurred occasionally. Mice which showed hemorrhagic manifestations usually died within the next 24 hours. The moribund suckling mouse was characterized by congested jugular veins, enlarged heart, progressive cyanosis, and decrease in surface temperature, but no manifestation of disease in the central nervous system was seen. Histologic examination of the bowel failed to reveal specific sites of bleeding but confirmed the congestive changes and showed vacuolar degeneration of the cytoplasm of mucosal cells

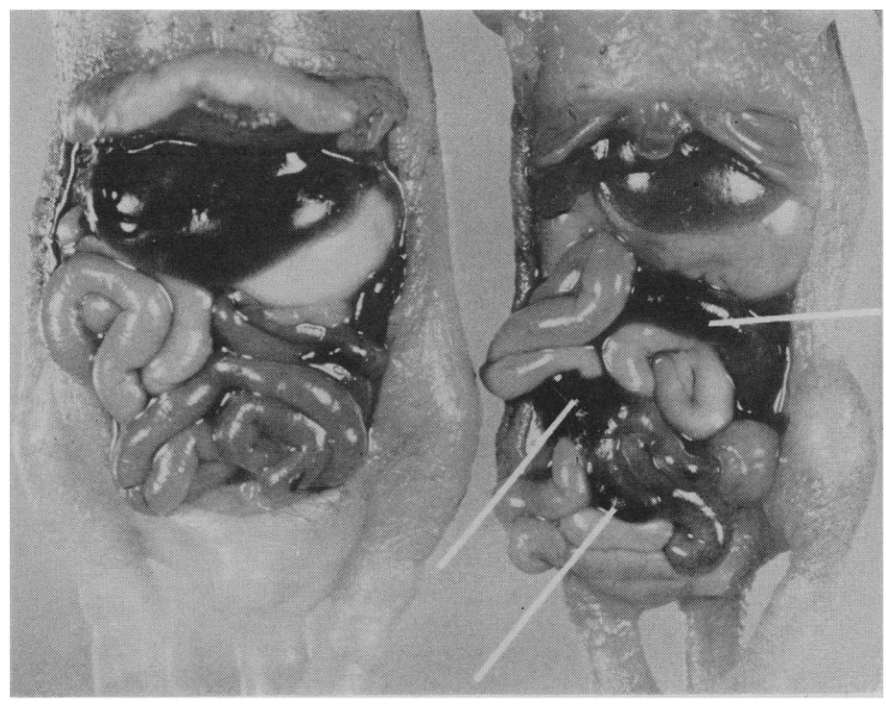

Fig. 1. Hemorrhage into isolated loops of bowel of 5-day old mouse, 72 hours after infection with KLA 16 virus (right). Normal abdominal viscera (left).

in the area of bleeding. No other characteristic lesions were seen regularly in the major viscera of infected mice. Bone marrows had normal complements of megakoryocytes and myeloid and erythroid elements. Normal hematopoietic tissues were present in liver and spleen. The cellularity and architecture of the thymus was undisturbed (*5*).

The mechanism for hemorrhage is at present poorly understood, although certain defects in the hemostatic mechanism have been observed. Platelet counts in the normal mouse 4 to 5 days old ranged from 640,000 to 1,200,000/mm^3 (average, 920,000/mm^3). In hemorrhagic mice infected with KLA 16, platelets were decreased to a range of 60,000 to 450,000/mm^3 (average, 220,000/mm^3). However, mice of the same age infected and moribund with Chikungunya virus in the 169th mouse passage maintained platelet counts near the normal range, 550,000 to 950,000/mm^3 (average, 700,000/mm^3). No other cellular element of peripheral blood was depressed. Rather, hemorrhagic mice showed leukocytosis to 20,000/mm^3 or more (normal 3000 to 5000/mm^3), with the increase due primarily to polymorphonuclear cells (65 to 90 percent). Whether this polymorphonuclear response resulted directly from KLA 16 virus infection, or whether it was the result of bacterial invasion through a damaged gastrointestinal mucosa, is unknown. Mice infected with KLA 16 have had consistently prolonged bleeding times (approximately 5×) and capillary tube venous clotting times (approximately 3×) compared with normal controls and with Chikungunya infected mice. Pooled plasma samples from groups of five litter mates with gastrointestinal hemorrhage were moderately icteric and possessed prothrombin activity 30 to 40 percent of normal controls.

Both 2 to 3 day old Syrian hamsters and albino rats (Wistar) are susceptible to hemorrhagic manifestations of KLA 16 virus infection. Intestinal hemorrhages in suckling hamsters appeared to be less severe than those seen in mice, but subcutaneous and subungual hemorrhages were prominent. On the other hand, the hemorrhagic disease in suckling rats was similar, but somewhat more severe than that seen in mice. The adult hamster, rat, guinea pig, and rabbit are, like the adult mouse, refractory to overt infection.

Overt hemorrhagic phenomena have not been observed in animals infected with TH 35 virus, and were seen only rarely with BaH 306 virus infection, although platelet counts, bleeding, and clotting times in mice infected with both of these viruses were abnormal. The changes, however, were less pronounced than those induced by KLA 16 infection. Since TH 35 and BaH 306 viruses had been subjected to a number of consecutive mouse passages, their failure to induce hemorrhage may be due to their mode of adaptation to rodents, or differences in methods for initial recovery, or both.

Hemorrhagic fever in Thailand is an acute infection of children and is characterized by fever, petechial hemorrhage, purpura, gastrointestinal bleeding, and shock (*2*). Its clinical manifestations are thus similar to the experimental animal disease. Current knowledge of the etiology, pathogenesis, and the explanation for the hemorrhagic tendency of Thailand hemorrhagic fever is incomplete; however, recorded alterations of the human hemostatic mechanism are compatible with those observed in mice. The limitation of recognizable disease in Thailand to infants and children suggests that the severity of the human disease, like its rodent counterpart, may be dependent upon age. While the extent of similarities remains undetermined, the suckling animal offers a unique and valuable tool for investigation of the pathologic process of this disease and for assessing the roles played by host factors such as growth and physiologic development in its manifestations.

The explanations for differences in the hemorrhage-producing capacities of the three immunologically identical virus strains is similarly unknown. All strains of Thai hemorrhagic fever virus may possess this hemorrhagic property, a thesis which can be evaluated only by critical study of additional recovered viruses. In this respect the hemorrhagic tendency of these viruses may be an important marker for rapid recognition of virus in the field. Certainly the hemorrhage-inducing property is not directly associated with the known antigenic composition of the virus, since immunologically identical strains differ in this capacity. The identification of this property with other marks of virus activity appears to be in order.

Finally, whether hemorrhage occurs in infected rodents may be dependent upon one or more interrelationships between virus and host. On one hand, source material might contain hemorrhage-producing variant in high concentration, or such a variant may propagate at rates favoring its selection. On the other, the characteristics of the host at the moment may be important in selecting different natural variants upon initial propagation, or in the recognition of hemorrhage-producing variants. Thus virus initially recovered in slightly older mice, or in other mouse strains, may have biologic characteristics different from the hemorrhage-producing variant (KLA 16) recovered in younger animals. The observed increase in the frequency of the hemorrhagic phenomenon after passage of virus from very young hemorrhagic mice tends to support the importance of host age and, further, implies that virus populations in nature are heterogeneous mixtures of hemorrhage- and nonhemorrhage-producing variants. These conditions may apply to other human hemorrhagic fever viruses. If this is so, further testing of the hypothesis should result in recovery of other hemorrhage-producing viruses in appropriate test animals.

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