Failure to Demonstrate Circulating Interferon During Incubation Period and Acute Stage of Transfusion-Associated Hepatitis (35379)

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(Introduced by R. M. Chanock)

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The presence of circulating interferon has been shown to parallel viremia in certain naturally occurring and experimentally induced virus infections of man and animals (1–5). This finding has suggested that demonstration of circulating interferon might serve as an indicator of the presence of viremia. Viral hepatitis is a disease presumed to be associated with one or more viruses which have thus far evaded isolation and characterization by existing laboratory techniques. Attempts to demonstrate interferon or virus-inhibiting substances in acute and convalescent sera from patients with viral hepatitis have been unsuccessful (6, 7). However, studies in which hepatitis virus was transmitted to human subjects led Krugman to conclude that viremia occurred prior to the onset of disease as well as during the acute phase (8); thus sera obtained during the acute and convalescent phases of hepatitis may be less infectious, therefore less likely to contain interferon, than sera obtained during the incubation period. The purpose of the present study was to attempt to detect interferon (and, thus, indirectly, viremia) in serum specimens collected throughout the incubation period as well as the acute phase of hepatitis and to determine if there was a relationship between the presence of hepatitis-associated antigen [HAA, Australia antigen (9–11)] and the detection of serum interferon.

Methods. Serum specimens were obtained as part of a prospective study of hepatitis in patients who underwent open-heart surgery at the National Heart and Lung Institute, NIH. Clinical and seroepidemiologic characterization of transfusion-associated hepatitis in these patients was reported previously (12, 13). For the purposes of this study, a transaminase value (SGPT or SGOT) of 80 Karmen units or greater (twice the upper limit of normal) was considered abnormal. Complement fixation (CF) tests for detecting HAA or antibody to HAA (anti-HAA) were performed as described previously (14).

Serum was separated from the clot and stored frozen at −70° until tested. Sera were not treated to inactivate any virus(es) which they might contain prior to testing. They were assayed for interferon at dilutions of 1:5 and 1:50; 1 ml of serum dilution was used/test.

Interferon was assayed by yield reduction of Sindbis virus in AH-1 cells (adult diploid-type cells from Mr. G. Gardiner, NIH Media Section). The interferon titer was recorded as the highest dilution of serum which inhibited the yield of Sindbis virus by 0.5 $\log_{50}$ during a single growth cycle; the yield of Sindbis virus was assayed by plaque formation on chick embryo fibroblasts. This method is a modification of the yield reduction method previously described (15). In an extensive series of comparative tests, the yield reduction method with Sindbis virus and AH-1 cells was found to be at least as sensitive as any other interferon assay method tested (S.

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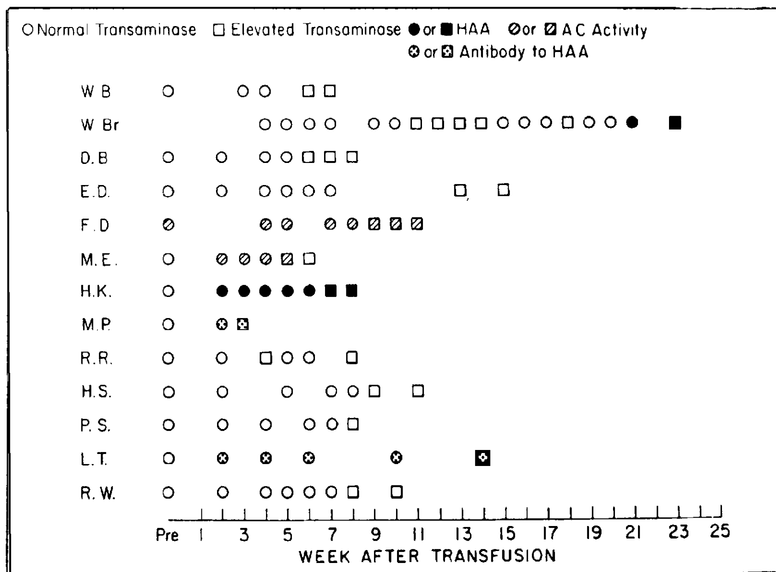

FIG. 1. Serial serum specimens, tested for interferon, from patients with transfusion-associated hepatitis.

Baron, personal communication). In other studies, the assay system was sufficiently sensitive to detect interferon in a 1:5000 dilution of spinal fluid from a patient with acute herpes virus encephalitis; a convalescent specimen was negative for interferon (S. Baron, personal communication). In the present study the titer of a control interferon preparation produced by human leukocytes *in vitro* averaged $10^{3.0} \pm 10^{0.5}$ in 6 sets of replicate assays.

Results. None of the serum specimens tested contained interferon detectable in a 1:5 dilution of the serum.

Seven of the 13 hepatitis patients were icteric; the maximum recorded transaminase values (SGOT:SGPT) for each patient ranged from 121:111 to 509:1476 (mean = 308:405).

Two of the 13 patients whose serum specimens were tested had HAA during the incubation period and acute phase of the illness (Fig. 1). Peak CF titers of HAA detected in these two patients were 1:2048 and 1:32, respectively. In addition, two patients had anticomplementary (AC) activity, an activity thought to represent, in some cases, immune complexes of HAA and anti-HAA (16) or other agent(s) and their respective antibodies (17, 18). In one patient, all serum specimens including the pretransfusion specimen had such AC activity (CF titer 1:2); AC activity in the serum specimens from the second patient was temporally related to the patient's hepatitis (maximum AC titer 1:8). Two patients developed anti-HAA prior to the onset of hepatitis but did not have detectable HAA. HAA, AC activity, or anti-HAA was not detected in serum specimens from the other patients tested.

Discussion. Published reports of attempts to detect interferon, or interferon-inducing capacity in serum specimens from patients with viral heapatitis have dealt with the examination of specimens from patients acutely ill with hepatitis or from persons who were known to have transmitted hepatitis to others (6, 7). However, none of the specimens tested were obtained during the incubation period or early acute stage of hepatitis when peak viremia is thought to occur. Furthermore, it was not determined whether the hepatitis so studied was positive for HAA or whether the serum specimens tested for interferon or interference-inducing capacity contained HAA. Finally, the test for detection of interference-inducing capacity employed by Taylor and Zuckerman (7) was not designed

to detect interferon and was therefore less sensitive for such detection than the test employed in the present study. Similarly, Wheelock *et al.* (6), although using a relatively sensitive interferon assay, tested sera at dilutions of 1:20 to 1:80, dilutions 4 to 16 times more dilute than the 1:5 dilutions we employed.

In the present study we have confirmed the failure to detect interferon in acute phase serum specimens from patients with hepatitis and, in addition, reported failure to detect interferon during all stages of the incubation period of viral hepatitis. Furthermore we have demonstrated that HAA, even in high titer (up to 1:2048 by CF) did not stimulate the production of detectable interferon *in vivo.* Evidence is accumulating that the presence of HAA in serum is indicative of hepatitis viremia [P. Holland, personal communication; (19, 20)]. If this is so, then one type of hepatitis virus, at least in the titers present in the specimens tested, did not appear to be an efficient inducer of interferon.

Anticomplementary activity has been found in acute phase sera from patients with HAA-positive or HAA-negative hepatitis. Such AC activity is thought to represent immune complexes of HAA and anti-HAA or of other hepatitis-related antigens and their respective antibodies (16–18). Sera containing such AC activity did not contain detectable interferon.

Wheelock listed five possible reasons why he did not detect interferon in acute convalescent serum specimens from patients with hepatitis: (i) inability of hepatitis virus(es) to stimulate efficiently the production of interferon; (ii) collection of serum specimens too late in the course of the disease; (iii) inefficient production of interferon by the patient because of liver disease; (iv) stimulation by hepatitis virus(es) of production of interferon-blocking substances; and (v) relative insensitivity of the interferon assay (6). We have demonstrated that late collection of serum specimens and inhibitory effect of liver disease on interferon production are unlikely explanations of failure to detect interferon since our specimens were collected during the incubation period and before chemical evidence of liver disease was detectable. Furthermore, it is unlikely that insensitivity of the interferon assay is an important reason for such failure; the interferon assays employed by Wheelock *et al.* (6) and by us were sensitive enough to detect interferon in specimens from patients infected with other viruses. We believe that the failure to detect interferon in serum specimens collected from hepatitis patients at a time when they are likely to be viremic is evidence that hepatitis viruses are poor stimulators of interferon production. Such a biological trait would be consistent with the prolonged or chronic viremia sometimes observed in viral hepatitis. Proof of this belief must await the isolation of hepatitis viruses and their characterization *in vitro.*

Summary. Ninety-six serial serum specimens collected prior to exposure and during the incubation period and acute phase of transfusion-associated hepatitis in 13 patients were tested for interferon. None was found. Two each of the patients had hepatitis-associated antigen, antibody to such antigen, or anticomplementary activity. Failure to detect interferon in hepatitis patients may be related to the tendency of viral hepatitis, in some cases, to become a prolonged or chronic disease.

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