LONG-INCUBATION POST-TRANSFUSION
HEPATITIS WITHOUT SEROLOGICAL
EVIDENCE OF EXPOSURE TO HEPATITIS-B
VIRUS
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Summary
An agent other than hepatitis-B (HB) virus seemed to be the cause of 36
(71%) of 51 cases of post-transfusion hepatitis identified
during prospective biweekly serological follow-up of 204 cardiovascular-surgery patients. The sera of
the 36 cases showed no evidence of the antigen or antibody response expected to accompany infection
by HB virus and to be detectable by the sensitive assays used. Incubation periods and clinical and epidemiological features were inconsistent with hepatitis A. Cytomegalovirus-associated seroconversion
was no more common among the HB-negative cases than among HB-positive cases or among patients who
did not develop hepatitis. The data suggest that a large proportion of long-incubation post-transfusion hepatitis is unrelated to hepatitis B and that control of post-transfusion hepatitis will require identification
of a hepatitis virus(es) type C.

Introduction
In a prospective study of 204 transfused patients we could find no evidence for the value of "convalescent" gamma-globulin, obtained from donors with a history of hepatitis or jaundice, as prophylaxis against post-transfusion hepatitis. Since globulin administration did not seem to affect the incidence or course of hepatitis, whether or not it was serologically related to type-B infection, the data from the group receiving "convalescent" globulin and the control group could be pooled. This provided an opportunity to evaluate the serological and clinical response of patients who developed post-transfusion hepatitis and to examine the role of hepatitis-B (HB) virus in the aetiology of post-transfusion hepatitis.

Patients and Methods
Patients
299 patients undergoing surgery at New York University Hospital from May, 1969, through August, 1972, were enrolled into this study. 204 completed the twenty-four week follow-up period (hepatitis was not the cause of any patient's death) determined necessary to detect a serological response to HB virus or development of hepatitis. Among the 204 patients, 57% were males, and the average age for both sexes was fifty-three years (range twenty-one to seventy-nine years). Almost all patients were White and came from middle or upper-middle socioeconomic backgrounds. Most of them underwent cardiovascular surgery and had presented with a history of arteriosclerosis (103 patients) or rheumatic heart-disease (90 patients). A pretransfusion blood-sample was drawn, and patients were followed up for four months postoperatively with biweekly blood-samples and then with monthly samples for the final two months. Among the patients who completed follow-up, an average of eleven blood-samples per patient was drawn over a six-month period; eleven or more samples were available from 91% of patients. These sera were tested in the virus laboratory of the New York Blood Center for hepatitis-B surface antigen (HB,Ag) or antibody (anti-HB,); and serum-transaminases were measured. Patients were encouraged to see their family physician or the study clinician if their serum-transaminase became high, if HB,Ag developed, or if the study nurse noted jaundice and/or clinical symptoms of hepatitis. At such time additional liver-function tests were done.

Donor Blood
3684 units were transfused to the 204 patients who completed the follow-up period—an average of 18 units per patient with a range of 2—102 units. 149 patients received all transfusions within a week of surgery. 24% of donor blood came from commercial sources. Before transfusion, 91% of donor blood (99% of all volunteer blood and 67% of all commercial blood) was tested for HB,Ag by agar-gel diffusion or counterelectrophoresis, and the positive bloods were eliminated.

Gamma-globulin
Patients enrolled in the trial were randomly assigned to receive either an albumin placebo (102 patients) or "convalescent" immune globulin obtained from American Red Cross donors with a history of hepatitis or jaundice two or more years before donation (93 patients). An additional 9 patients were followed who inadvertently received standard immune-globulin. When a sensitive test for anti-HB became available, it was found that the convalescent immune-globulin had an antibody titre (1/4 by passive haemagglutination) similar to that of most conventional globulin preparations available at that time.

Laboratory Tests
HB,Ag was sought in sera by agar-gel diffusion 4 and/or counterelectrophoresis and by the 'Austria I' radioimmunoassay (R.I.A.) according to the instructions of the manufacturer (Abbott Laboratories, Chicago). HB,Ag specificity of positive R.I.A. results was determined by attempted neutralisation with anti-HB, before retesting by R.I.A.

Anti-HB, was determined by a passive-haemagglutination assay using a modification 5 of the method of Vyaz and Shulman, and the specificity of positive results was determined by addition of HB,Ag. At the end of this study all serum sera from any patient who made an anti-HB response were retested in consecutive order on the same day.

Serum-glutamic-pyruvic-transaminase (s.g.p.t.) levels were measured by the kinetic spectrophotometric method of Wroblewski and LaDue.

Cytomegalovirus complement-fixing antibody levels were determined in sera by the microtitre complement-fixation technique in the virus laboratory, New York City Department of Health. Before testing, specimens were coded and inactivated for thirty minutes at 56°C. Antigen (AD 169 strain) was obtained from Microbiological Associates (Bethesda, Maryland).

After initial testing all sera were held at −70°C.

Terminology
All cases with transaminase abnormalities were reviewed (in the absence of serological data) by a panel of clinicians.
to exclude cases likely to have causes of liver-function abnormality other than post-transfusion hepatitis. "Hepatitis" was defined as two or more consecutive elevations of serum-transaminase above 60 Karmen units (or, if tested by an outside laboratory, 2.5 times the upper limit of normal for that laboratory) fourteen or more days apart and from fourteen to one hundred and eighty days post-transfusion. For "icteric hepatitis" the criteria were the same as above plus a serum-bilirubin of 2.5 mg. per 100 ml. or more; or jaundice noted by patient’s physician, study clinician, or study nurse. For patients with valve replacements, the definition of icteric hepatitis was 2.5 times baseline bilirubin or > 5 mg. per 100 ml. if no baseline values were available. The “incubation period” was defined as the period from transfusion to first transaminase elevation or jaundice, whichever came first. Patients who did not receive all transfusions within seven days of surgery were excluded from all calculations of incubation period but not from the remainder of the analysis.

**TABLE I—FREQUENCY OF HB SEROLOGICAL RESPONSE IN CASES OF POST-TRANSFUSION HEPATITIS**

<table>
<thead>
<tr>
<th>Clinical response</th>
<th>No. with a serological HB response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbAg only</td>
</tr>
<tr>
<td>Icteric hepatitis</td>
<td>21</td>
</tr>
<tr>
<td>Anicteric hepatitis</td>
<td>30</td>
</tr>
<tr>
<td>No hepatitis</td>
<td>153</td>
</tr>
</tbody>
</table>

**TABLE II—CLINICAL AND SEROLOGICAL RESPONSE IN 13 PATIENTS WHO ACQUIRED HB Ag**

<table>
<thead>
<tr>
<th>Clinical response</th>
<th>No.</th>
<th>Mean onset (wk.)</th>
<th>Mean peak R.I.A. (units)</th>
<th>Mean on-going positivity</th>
<th>Median titre (wk.)</th>
<th>No. with antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis*</td>
<td>6</td>
<td>9.7 (±6-26)</td>
<td>24.5 (±21-54)</td>
<td>5</td>
<td>7 (±5)</td>
<td>4</td>
</tr>
<tr>
<td>No hepatitis</td>
<td>7</td>
<td>13.5 (±9-61)</td>
<td>6.0 (±3-6)</td>
<td>0</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Significance</td>
<td>N.S.</td>
<td>P &lt; 0.05</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Icteric and anicteric.
† 1 case excluded because not all transfusions were given within a week of surgery.
‡ 2 cases excluded because not all transfusions were given within a week of surgery.

was defined as ≥1/16 titre in the pretransfusion serum with less than a 4-fold rise in titre.

Cytomegalovirus antibody seroconversion was defined as a 4-fold or greater rise in antibody titre between the pretransfusion and the four to six month post-transfusion specimen.

**Results**

**Treatment Groups**

51 of the 204 patients who completed follow-up developed hepatitis, 21 with jaundice. Of the patients who received convalescent immune-globulin, 26% developed hepatitis. 25% of the group that received the albumin placebo and 22% of the group receiving conventional globulin also developed hepatitis. The time of onset of hepatitis was similar in all three groups.

**Hepatitis Type B**

Only 15 hepatitis cases gave evidence of exposure to hepatitis-B virus as determined by development of HbAg and/or an anti-HB response. An additional 25 patients had a hepatitis-B response but did not develop hepatitis. In all, 33% of icteric cases, 2% of anicteric cases, and 16% of patients without hepatitis were found to have serological evidence of hepatitis-B-virus exposure (table 1). Examples of the serological reaction patterns in patients with type-B hepatitis are shown in fig. 1.

**SEROLOGICAL RESPONSES**

Detection of HB Ag in the second or later post-transfusion serum was considered to indicate evidence of HB infection rather than passive transfer of HB Ag from transfused blood.

Anti-HB was thought to be passively acquired from transfused blood where the titre was <1/8 in the pretransfusion specimen and peaked in the first post-transfusion specimen to >1/8 and <1/512. A primary antibody response was defined by a pretransfusion titre of <1/8 and an 8-fold or greater rise in titre more than four weeks post-transfusion. Anamnestic or secondary antibody response was defined as 8-fold or greater rises in titre to >1/512 within four weeks of transfusion. Pre-existing (or pretransfusion) antibody without hepatitis-B response
Of the patients with an HB response without hepatitis, 15 gave evidence of a primary exposure (i.e., they developed HB_Ag and/or a primary anti-HB_Ag response) and the remaining 10 made anamnestic responses. Examples of patients with serological evidence of HB exposure without hepatitis are shown in fig. 2. None of the patients who developed an anamnestic antibody response developed antigenemia.

Altogether 13 patients developed detectable HB_Ag (table IV). None of these patients became long-term carriers. 6 of these patients also developed hepatitis. A comparison of patients with antigenemia shows that among those who developed hepatitis, the mean onset of antigenemia was earlier, the mean peak R.I.A. was higher, and the median duration of antigenemia was longer. However, only the difference in mean peak R.I.A. values was statistically significant (P < 0.05). In general, when a patient developed both antigen and a primary antibody response, antibody was detectable only after antigen had disappeared.

Since the mean duration of antigenemia was longer in hepatitis patients, the mean time of onset of antibody occurred later in these patients.

**Hepatitis without Evidence of HB Exposure**

36 of the 51 cases of hepatitis (71%) did not give evidence of HB exposure (table III). Jaundice occurred in 47% of cases of HB hepatitis with and in 39% of hepatitis cases without HB response. The mean incubation period, mean peak S.G.P.T., and median duration of transaminase elevation were comparable. The clinical course of 3 patients with HB-negative hepatitis is shown in fig. 3.

9 hepatitis cases had an incubation period of four-teen to forty-five days; 2 of these were HB positive (in both of these cases onset occurred at forty-two days). A slightly but not significantly higher proportion (9/25) of cases with an incubation period of more than forty-five days had detectable HB_Ag and/or anti-HB_Ag.

There was no evidence of delay in onset of HB-negative hepatitis among recipients of globulin: the mean onset was at fifty-six days for the group receiving convalescent globulin and forty-four days for the group receiving the placebo. The clinical course of HB-negative hepatitis was also similar in the two treatment groups.

The possible role of cytomegalovirus in the aeti-
logy of non-B hepatitis was examined. Sera from 47 cases of hepatitis (15 type B) and 82 cases without hepatitis were tested for antibody to cytomegalovirus. A 4-fold or greater rise in antibody titre was found in 33% of the hepatitis cases with evidence of HB exposure, 19% of hepatitis cases without demonstrable HB response, and 24% of patients without hepatitis. We concluded that cytomegalovirus was not responsible for the HB-negative cases of post-transfusion hepatitis. The possible role of Epstein Barr (E.B.) virus in the aetiology of non-B hepatitis was also considered. Most transfusion recipients have pre-existing E.B. antibody, and seroconversion is not common and has not been correlated with non-B hepatitis. It is therefore unlikely that E.B. virus plays an important role in post-transfusion hepatitis.

**Effect of Passively Acquired and Pretransfusion Antibody**

The clinical course of patients who acquired anti-HB from transfused blood and patients with pretransfusion antibody was examined to determine whether past exposure to HB virus or passively acquired antibody would protect against development of or attenuate the course of hepatitis. The clinical response of patients known to have been exposed to HB infection on the basis of their serological response is summarised in Table IV. Although no evidence of protection is evident in those who received anti-HB, passively in transfused blood, the data do suggest a reduction in the incidence of hepatitis in patients with anti-HB, before transfusion.

The question of whether prior exposure to HB virus affords immunity is further examined in Table V. Among 27 patients who made HB,Ag and/or primary anti-HB, response, 12 (44%) developed hepatitis. Among 13 patients with an anamnestic antibody response, 3 (23%) developed hepatitis. In the latter group there were no cases of jaundice. 12 out of 13 patients who made an anamnestic response had detectable levels of antibody in their pretransfusion serum.

**Discussion**

**Aetiology of Post-transfusion Hepatitis**

It is probable that most individuals exposed to blood containing HB virus respond serologically, antigen and/or primary or anamnestic antibody response being detectable with the sensitive assays now available. In this study 10 out of 11 recipients of blood containing HB,Ag gave serological evidence of HB exposure. Similar results have been reported when sera from recipients of blood containing HB,Ag were tested serially; an HB response was detectable in 34/35 and 119/123 such recipients. Considering the high efficiency of the assays used, it seems probable therefore that most of the 36 HB-negative hepatitis cases in our series were not due to infection with HB virus.

It might be argued that the lack of serological response was due to exposure to doses of HB virus so low as to give rise to hepatitis without development of antigenemia or antibody seroconversion. However, Barker et al. found that an inoculum of an undiluted ictericogenic plasma pool (in which HB,Ag was barely detectable by complement fixation and was not detectable by agar-gel diffusion) produced hepatitis in 22 and antigenemia in 25 of 37 recipients; and inoculation of 10⁴ to 10⁶ dilutions of the same material caused 7 of the 15 recipients to develop an antigenic response even though none developed hepatitis.

In our series 3 patients had anti-HB (titre 1/45 to 1/400) in their serum before transfusion. These patients developed hepatitis (2 with icterus) seven to twelve weeks post-transfusion, but did not develop the anamnestic response which would be expected if they had been re-exposed to HB,Ag (fig. 3, middle panel). It would seem unlikely that these cases of hepatitis could have been due to reinfection with HB virus. A different approach to the detection of HB-virus infection is to look for antibody to the core antigen of the Dane particle (anti-HBc). We have tested paired sera (the pretransfusion specimen and a specimen drawn four to six months after surgery) from 21 of our hepatitis patients for anti-HBc by indirect immunofluorescence: we found anti-HBc in all of 6 patients with HB-positive hepatitis but not in 14 of 15 patients with HB-negative hepatitis.

There have been several other reports of HB-negative post-transfusion hepatitis. Most of these cases have been thought to be hepatitis A. However, we found no significant difference between the incubation periods of the 15 cases of HB-positive hepatitis and the 36 cases with hepatitis of unknown aetiology. This argues against an important role for the hepatitis-A agent in these cases. Furthermore, no intraspecific transmission of overt hepatitis was observed in the households of the HB-negative cases, as would be expected in type-A infections. This conclusion also accords with the finding that the non-B hepatitis wa
not modified by administration of gamma-globulin. Parcell has tested 28 cases of non-B post-transfusion hepatitis for anti-HB antibody by immune electron microscopy; none developed a rise in titre.

It might be argued that non-B hepatitis is not an infection but a disturbance of liver function secondary to underlying or intercurrent non-viral disease. However, in this series the risk of non-B hepatitis was ten times higher among recipients of blood obtained from commercial sources than among those given blood from volunteer donors; it is unlikely that the donor source could affect the incidence of non-viral hepatitis.

Characterisation of Primary HB Infection

Characterisation of HB infection is difficult because of the great variation in host response. This may explain the disparity in reports of the clinical and serological response of individuals exposed to HB virus. In our series hepatitis developed in only 12/27 (44%) patients with an apparent primary exposure to HB virus (i.e., patients who developed HB_Ag and/or a primary antibody response). In contrast, other prospective follow-up studies have reported hepatitis in 57-89% of patients who developed antigen or a primary antibody response. The higher frequency of hepatitis among patients with a primary HB response in these studies may in part be related to more careful follow-up of patients who became clinically ill. As noted above, antigen is not readily detectable in patients without biochemical evidence of liver disease since antigen is produced in lesser amount and for a shorter period of time.

We found HB_Ag in 33% of patients who made an HB response. In other series antigen has been found in 22-98% of such patients. The sensitivity of assays used to detect HB response must be considered in a comparison of different reports, since antibody, especially a primary antibody response, is not readily detectable with less sensitive methods, and the percentage of patients with antigen will depend upon the total number of exposed cases detected.

A review of follow-up studies of HB-exposed individuals indicates that there may be an increased risk of developing detectable levels of antigen in younger age-groups: of 40 children exposed to MS-2 containing serum, 39 developed antigen; Barker et al. reported that antigen was detected in 93/123 of the young adults who received an inoculum containing HB_Ag. In contrast, in two studies of transfused patients with mean ages of forty-seven and over fifty, antigen was detected in only 22% of patients who made a serological HB response. Likewise, in our series in which the average age of patients was fifty-three, comparatively few patients developed antigen.

Immunity in HB Infection

In 1946 Neefe et al. reported that none of 9 volunteers developed hepatitis after reinoculation with plasma containing "a causative agent of serum hepatitis" although they had all developed hepatitis after their initial exposure. The absence of hepatitis or antigenemia in patients who make an anamnestic anti-HB response has been reported. However, hepatitis has been observed in a small number of patients who had anti-HB, before exposure. Barker et al. have reported 23 cases in which an anamnestic HB response was followed by development of hepatitis and/or antigenemia; 5 of these patients became chronic antigen carriers.

It has not been determined whether exposure to one strain of HB_Ag will protect against reinfection with strains carrying other subtype antigens, or whether passive transfer (via transfusion of blood containing anti-HB, or administration of hepatitis B immune-globulin) of one strain of anti-HB, will protect against infection with other subtype antigens. Development of antigenemia or type-B hepatitis after passive antibody transfer or secondary antibody response may thus be due to infection with a previously unexperienced antigenic specificity. The amount and virulence of the exposure dose must also be considered in determining the protective effect of passive or active antibody.

Our results suggest that prior exposure to HB infection provides some degree of immunity to reinfection: only 3/12 patients with anamnestic HB, responses developed hepatitis (all anicteric) in comparison to 12/27 patients (7 icteric) who developed antigenemia and/or primary antibody responses. However, we found that passively acquired anti-HB, in quantities similar to those which would be produced by an injection of hepatitis B immune-globulin, failed to protect 3 of 4 HB-exposed patients from development of hepatitis.

Significance of HB-negative Hepatitis

It can be argued that a disproportionately high number of non-B hepatitis cases were observed because 91% of donor blood was prescreened and HB_Ag-containing units were eliminated. Analysis of the frequency of counterelectroforesis detectable HB_Ag among volunteer and commercial donors serving the greater New York area during the period of this study indicates that an additional 103 antigen-containing units would have been transfused if the blood had not been prescreened. Since only 37.5% of patients in this study with an HB serological response developed hepatitis, it is therefore estimated that only 4.3% of the recipients of the positive units would have developed hepatitis. Thus, on the basis of this estimate, the proportion of cases attributable to HB virus would not have increased significantly.

The course of clinical illness in patients with HB-positive and HB-negative hepatitis did not differ. In contrast, Glocke has reported findings correlating between severity of clinical illness and HB-positive hepatitis. In a review of a large number of hospital cases of post-transfusion and "shared needle" hepatitis, it was reported that 76% were found by complement fixation to have HB_Ag in acute-phase sera. Furthermore, all sera from these patients have been tested and the sera been tested by a more sensitive assay, antigen would have been detected in a higher proportion of cases. Thus, although most cases of hepatitis with a severe clinical course appear to be predominantly type B, careful prospective follow-up of transfused patients reveals...
that the major proportion of all hepatitis cases are of unknown etiology.

The fact that non-B hepatitis cases are less frequently associated with serious acute illness does not imply that such cases are of lesser importance. Long-term complications of acute hepatitis-B infection, such as chronic hepatitis, cirrhosis, and hepatoma, have been reported to follow mild anicteric infections more frequently than in severe icteric cases; consideration must thus also be given to the possibility that non-B hepatitis may play a role in the etiology of some forms of chronic liver disease.

Our findings imply that a substantial proportion of post-transfusion hepatitis cases is caused neither by HB virus nor hepatitis A agent, and suggest the existence of an additional virus(es), hepatitis type C.

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REFERENCES


22. Purcell, R. H. Paper read at the 6th annual scientific symposium of the American Red Cross, held in Washington, D.C., in May, 1974.


REFERENCES continued at foot of next column

RELATION BETWEEN ESCHERICHIA COLI
KI CAPSULAR POLYSACCHARIDE ANTIGEN
AND CLINICAL OUTCOME IN NEONATAL
MENINGITIS

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Summary

The clinical outcome in fifty-seven infants with Escherichia coli meningitis was analysed with respect to the presence or absence of K1 capsular polysaccharide antigen. Mortality and morbidity in E. coli K1 meningitis were significantly greater than in meningitis caused by E. coli non-K1 strains. The amount of K1 antigen and length of time K1 antigen was present in serum and cerebrospinal fluid, as measured by countercurrent immunoelectrophoresis, were directly related to clinical outcome. E. coli K1 strains were more virulent in mice than non-K1 strains, and the lethal dose of K1 strains from infants who died was significantly lower than those values from infants who survived E. coli K1 meningitis.

Introduction

Escherichia coli has been the most common cause of neonatal purulent meningitis for the past twenty years.

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DR PRINCE AND OTHERS: REFERENCES—continued


