LONG-INCUBATION POST-TRANSFUSION HEPATITIS WITHOUT SEROLOGICAL EVIDENCE OF EXPOSURE TO HEPATITIS-B VIRUS

ALFRED M. PRINCE BETSY BROTMAN
GEORGE F. GRADY WILLIAM J. KUHNS
CHARLES HAZZI RICHARD W. LEVINE
STEPHEN J. MILLIAN

Laboratory of Virology, New York Blood Center; State Laboratory Institute, Massachusetts Department of Public Health, Boston; Departments of Pathology and Medicine, New York University School of Medicine; and Virus Laboratory, New York City Department of Health

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.[1] 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 ætiology 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 socio-economic 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_sAg) or antibody (anti-HB_s), 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_sAg 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_sAg 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).[1] An additional 9 patients were followed who inadvertently received standard immune-globulin. When a sensitive test for anti-HB_s became available,[2] it was found that the convalescent immune-globulin had an antibody titre (1/4 by passive hæmagglutination) similar to that of most conventional globulin preparations available at that time.[3]

Laboratory Tests

HB_sAg was sought in sera by agar-gel diffusion [4] and/or counterelectrophoresis [5] and by the 'Ausria I' radioimmunoassay (R.I.A.)[6] according to the instructions of the manufacturer (Abbott Laboratories, Chicago). HB_sAg specificity of positive R.I.A. results was determined by attempted neutralisation with anti-HB_s before retesting by R.I.A.[7]

Anti-HB_s was determined by a passive-hæmagglutination assay using a modification [2] of the method of Vyas and Shulman,[8] and the specificity of positive results was determined by addition of HB_sAg.[2] At the end of this study all serial sera from any patient who made an anti-HB_s 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.[9]

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

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

Clinical response	No.	No. with a serological HB response				
		HB_sAg only	HB_sAg and primary anti-HB_s	Primary anti-HB_s only	Secondary anti-HB_s only	Total
Icteric hepatitis ..	21	2	2	3	0	7 (33%)
Anicteric hepatitis	30	0	2	3	3	8 (27%)
No hepatitis ..	153	2	5	8	10	25 (16%)

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 $\geqslant 5$ 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.

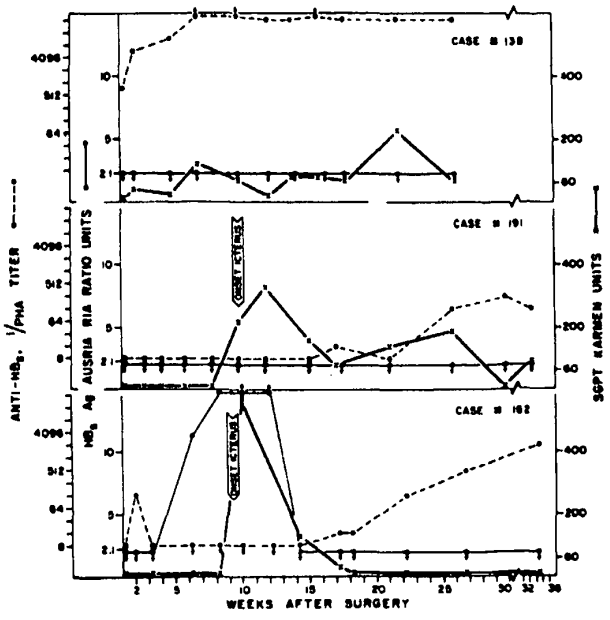

Fig. 1—Patients with hepatitis and with evidence of HB exposure.

Case 138 (top): anamnestic antibody response, long-incubation anicteric hepatitis, and no detectable HB_sAg.

Case 191 (middle): icteric hepatitis with an onset of 9½ weeks, a primary antibody response, and no detectable HB_sAg.

Case 162 (bottom): antigenæmia detectable 6 weeks post-transfusion, icteric hepatitis with an onset of 9 weeks, passively transfused anti-HB_s detectable immediately post-transfusion, and a primary anti-HB_s response.

Serological Responses

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

Anti-HB_s was thought to be passively acquired from transfused blood where the titre was $<1/8$ in the pre-transfusion specimen and peaked in the first post-transfusion specimen to $>1/8$ and $\leqslant 1/512$. 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 was defined as $\geqslant 1/16$ titre in the pretransfusion serum with less than a 4-fold rise in titre.

TABLE II—CLINICAL AND SEROLOGICAL RESPONSE IN 13 PATIENTS WHO ACQUIRED HB_sAg

Clinical response	No.	Antigen response				Primary antibody response	
		Mean onset (wk.)	Mean peak R.I.A. (ratio units)	No. on agar-gel diffusion positive	Median duration (wk.)	No. with antibody	Mean onset (wk.)
Hepatitis*	6	9·7 (±6·2)†	24·0 (±11·4)	5	7·6	4	23·2 (±6·4)
No hepatitis	7	13·5 (±8·6)‡	6·0 (±3·6)	0	1·0	5	14·2 (±7·4)‡
Significance		N.S.	$P < 0·05$	..	..	..	N.S.

* 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.

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.[1]

Hepatitis Type B

Only 15 hepatitis cases gave evidence of exposure to hepatitis-B virus as determined by development of HB_sAg and/or an anti-HB_s response. An additional 25 patients had a hepatitis-B response but did not develop hepatitis. In all, 33% of icteric cases, 27% of anicteric cases, and 16% of patients without hepatitis were found to have serological evidence of hepatitis-B-virus exposure (table I). Examples of the serological reaction patterns in patients with type-B hepatitis are shown in fig. 1.

Of the patients with an HB response without hepatitis, 15 gave evidence of a primary exposure (i.e., they developed HB_sAg and/or a primary anti-HB_s 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 antigenæmia.

Altogether 13 patients developed detectable HB_sAg (table II). None of these patients became long-term carriers. 6 of these patients also developed hepatitis. A comparison of patients with antigenæmia shows that among those who developed hepatitis, the mean onset of antigenæmia was earlier, the mean peak R.I.A. was higher, and the median duration of antigenæmia 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 antigenæmia 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-

TABLE III—COMPARISON OF HEPATITIS CASES WITH AND WITHOUT EVIDENCE OF EXPOSURE TO HB_sAg

Type of case	No.	% Icteric	Mean incubation period (wk.)	Mean peak S.G.P.T.	Median duration S.G.P.T. ≥ 60 units (wk.)
Hepatitis with serological evidence of HB exposure	15	47	10·4 (±4·9)*	318 (±280)	8·9
Hepatitis without serological evidence of HB exposure	36	39	8·0 (±2·7)†	259 (±131)	10·5
Total	51	41	8·8 (±3·6)	277 (±186)	9·9

* 4 cases not included since not all transfusions were given within a week of surgery.
† 13 cases not included since not all transfusions were given within a week of surgery.

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_sAg and/or anti-HB_s.

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 fifty-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 ætio-

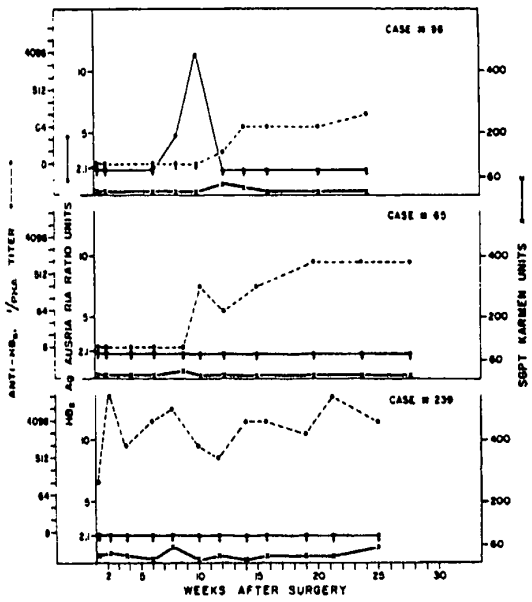

Fig. 2—Patients without hepatitis but with evidence of HB exposure.

Case 98 (top): detectable antigen at 8 weeks post-transfusion and a primary antibody response.

Case 65 (middle): only a primary antibody response at 10 weeks post-transfusion.

Case 239 (bottom): anamnestic antibody response only.

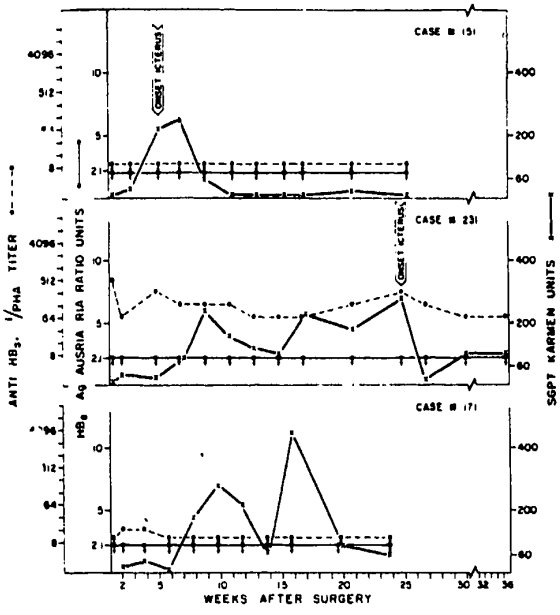

Fig. 3—Patients with hepatitis but with no evidence of HB exposure.

Case 151 (top): icteric hepatitis developing at 5 weeks post-transfusion.

Case 231 (middle): icteric hepatitis developing at 7 weeks, pretransfusion anti-HB_s, and no significant change in antibody titre after transfusion.

Case 171 (bottom): anicteric hepatitis developing at 8 weeks.

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 detectable 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 ætiology of non-B hepatitis was also considered. Most transfusion recipients have pre-existing E.B. antibody,[10] 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_s 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_s passively in transfused blood, the data do suggest a reduction in the incidence of hepatitis in patients with anti-HB_s 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_sAg and/or primary anti-HB_s 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.

TABLE IV—EFFECT OF PASSIVELY ACQUIRED OR PRETRANSFUSION ANTI-HB_s ON DEVELOPMENT OF HEPATITIS IN HB-EXPOSED PATIENTS

Patients with	No.	No. developing HB_sAg and/or anti-HB_s	No. developing hepatitis		
			Icteric	Anicteric	Total
Passively acquired anti-HB_s only	26	4	2	1	3 (*75%*)
Pretransfusion anti-HB_s only	26	12*	0	2	2 (*17%*)
Without anti-HB_s when transfused	152	24	5	5	10 (*42%*)

* Although 13 patients gave evidence of prior exposure to HB virus by making an anamnestic anti-HB_s response, only 12 of these patients had detectable antibody in their pretransfusion serum.

TABLE V—EFFECT OF ANAMNESTIC ANTI-HB_s RESPONSE IN PATIENTS WITH SEROLOGICAL EVIDENCE OF HB EXPOSURE

HB serological response	No. patients at risk	No. with anti-HB_s in pretransfusion sera	Hepatitis		
			Icteric	Anicteric	Total
HB_sAg and/or primary anti-HB_s	27	0	7 (*26%*)	5 (*19%*)	12 (*44%*)
Anamnestic anti-HB_s	13	12	0	3 (*23%*)	3 (*23%*)

Discussion

Ætiology 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_sAg gave serological evidence of HB exposure.[11] Similar results have been reported when sera from recipients of blood containing HB_sAg were tested serially; an HB response was detectable in 34/35 [12] and 119/123 [13] 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 antigenæmia or antibody seroconversion. However, Barker et al. found that an inoculum of an undiluted icterogenic plasma pool (in which HB_sAg was barely detectable by complement fixation and was not detectable by agar-gel diffusion) produced hepatitis in 22 and antigenæmia in 25 of 37 recipients; and inoculation of 10^{-5} to 10^{-7} dilutions of the same material caused 7 of the 15 recipients to develop an antigen response even though none developed hepatitis.[14]

In our series 3 patients had anti-HB_s (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_sAg (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 [15,16] is to look for antibody to the core antigen of the Dane particle (anti-HB_c). 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-HB_c by indirect immunofluorescence: we found anti-HB_c in all of 6 patients with HB-positive hepatitis but not in 14 of 15 patients with HB-negative hepatitis.[17]

There have been several other reports of HB-negative post-transfusion hepatitis.[18-21] 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 ætiology. This argues against an important role for the hepatitis-A agent in these cases. Furthermore, no intrafamilial 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 was

not modified by administration of gamma-globulin. Purcell [22] has tested 28 cases of non-B post-transfusion hepatitis for anti-HA 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 [11]: 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_sAg 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.[18,19,23–25] 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 amounts and for a shorter period of time.

We found HB_sAg in 33% of patients who made an HB response. In other series antigen has been found in from 22–98% of such patients.[13,18,19,23–27] 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 detected 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 [26]; Barker et al. reported that antigen was detected in 93/123 of the young adults who received an inoculum containing HB_sAg.[13] 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.[25,27] 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.[28] 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 antigenæmia in patients who make an anamnestic anti-HB_s response has been reported.[18,20,27,29] However, hepatitis has been observed in a small number of patients who had anti-HB_s before exposure.[23,26,30] Barker et al. have reported 23 cases in which an anamnestic HB response was followed by development of hepatitis and/or antigenæmia; 5 of these patients became chronic antigen carriers.[13]

It has not been determined whether exposure to one strain of HB_sAg will protect against reinfection with strains carrying other subtype antigens, or whether passive transfer (via transfusion of blood containing anti-HB_s or administration of hepatitis B immune-globulin [3]) of one strain of anti-HB_s will protect against infection with other subtype antigens. Development of antigenæmia 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 3/13 patients with anamnestic anti-HB_s responses developed hepatitis (all anicteric) in comparison to 12/27 patients (7 icteric) who developed antigenæmia and/or primary antibody responses. However, we found that passively acquired anti-HB_s, in quantities similar to those which would be produced by an injection of hepatitis B immune-globulin,[3] 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_sAg-containing units were eliminated. Analysis of the frequency of counterelectrophoresis-detectable HB_sAg among volunteer and commercial donors serving the greater New York area during the period of this study indicates that an additional 10·3 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, Gocke has reported finding a correlation between severity of clinical illness and HB-positive hepatitis.[19] In a review of a large number of hospital cases of post-transfusion and "shared needle" hepatitis,[31] it was reported that 76% were found by complement fixation to have HB_sAg in acute-phase sera. Futhermore, had sera from these patients been tested before onset of illness and had 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 ætiology.

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 severe icteric cases[31]; consideration must thus also be given to the possibility that non-B hepatitis may play a role in the ætiology 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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Requests for reprints should be addressed to A. M. P., New York Blood Center, 310 East 6th Street, New York, N.Y. 10021, U.S.A.

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RELATION BETWEEN ESCHERICHIA COLI K1 CAPSULAR POLYSACCHARIDE ANTIGEN AND CLINICAL OUTCOME IN NEONATAL MENINGITIS

GEORGE H. MCCRACKEN, JR. LARRIE D. SARFF
MARY P. GLODE SUSAN G. MIZE

Departments of Pediatrics and Medical Computer Science, University of Texas, Southwestern Medical School, Dallas, Texas, U.S.A.

MARK S. SCHIFFER JOHN B. ROBBINS

Developmental Immunology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, U.S.A.

EMIL C. GOTSCHLICH

Rockefeller University, New York, U.S.A.

IDA ORSKOV FRITS ORSKOV

International Escherichia Centre, World Health Organisation, Copenhagen, Denmark

COOPERATIVE NEONATAL MENINGITIS STUDY GROUP*

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

* Charles A. Alford (Birmingham), Bernard Boxerbaum (Cleveland), Ernesto Calderon (Mexico City), Moses Grossman (San Francisco), Marc Gurwith (Winnipeg), Jerome Klein (Boston), Sheldon Korones (Memphis), Irwin Light (Cincinnati), Melvin Marks (Montreal), George H. McCracken (Dallas), Richard Michaels (Pittsburgh), Susan Mize (Dallas), Andre Nahmias (Atlanta), James Overall (Salt Lake City), Herman Risemberg (Baltimore), Hugo Trujillo (Medellin), Catherine M. Wilfert (Durham), Harry T. Wright (Los Angeles), and Richard Zachman (Madison).

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