

developed antigenaemia (see table IV), 2 symptomless whilst immunosuppressed and 1 acute clinical hepatitis when not immunosuppressed. None of these patients had anti-HBs nor did it appear subsequently in them before or after antigenaemia. 3 patients developed anti-HBs at concentrations detectable by I.E.O.P., 1 soon after transplantation with a kidney from an antigen-positive donor; for the others no cause could be found.

Discussion

From these results the use of antigen containing HbC seems to be a valid method for the detection of antibody to H.B.V. by I.E.O.P. if the precipitin lines are found to contain HbC antigen/antibody aggregates. The 6.1% frequency of anti-HbC in this series of transplant patients is greater than in blood-donors where Cohen⁷ found 7 in 929 (0.7%) and Hoofnagle et al.² found 1 in 100 volunteer donors in the U.S.A.

Only 17 of the 23 patients with anti-HbC also had anti-HBs detectable in their sera. The number of patients with anti-HBs and not anti-HbC has not yet been determined. However, it is interesting to compare subsequent changes in those with anti-HbC alone and those with both antibodies since in each instance previous infection is inferred.

In 3 of the 6 patients with anti-HbC alone immunosuppression was associated with the onset of antigenaemia eight, eleven, and more than fifty-two weeks later. This implies reactivation of latent infection if anti-HbC is evidence of prior infection and would be analogous to the reactivation of other viruses, such as herpes viruses, which is now well documented. Hoofnagle et al.² considered the possibility that persistence of anti-HbC could be an indication of continued viral replication and our results would fit this hypothesis. In 2 of these patients the level of anti-HbC fell to an undetectable amount after the start of immunosuppression and only reappeared with the onset of antigenaemia, which is a parallel finding to that of anti-HBs antibody in immunosuppressed leukaemic patients⁸ and has also been seen in the virus antibodies in cytomegalovirus (C.M.V.) infections.⁹⁻¹¹ In the group of 17 patients with both antibodies no cases of antigenaemia occurred. The only change that was noted was the increased production of anti-HBs in 3. This may not indicate that the presence of anti-HBs necessarily removes the possibility of antigenaemia since Wands et al.⁸ found that 5 of 17 leukaemics with antibody became antigenaemic when they were immunosuppressed but our series suggests that the probability is much less in transplant patients.

In the remaining 357 without anti-HbC initially a further parallel with C.M.V. infection is also provided by the 2 patients who became antigenaemic. They are assumed to have had primary infections and they did not produce detectable antibody after they became antigenaemic. This has frequently been found in C.M.V. infections where complement-fixing antibody did not appear in patients considered to have primary infections whilst immunosuppressed (Craighead,⁹ 1 case; Nagington,¹⁰ 6 of 10; Pien et al.¹² 9 of 11; Fiala et al.¹¹ 4 of 11).

If our observations are confirmed hepatitis B must be regarded as a reactivable infection of importance in the management of transplant patients. This does not imply that prospective transplant patients should be screened

for anti-HbC in order to exclude them. This would be unwarranted as it is likely that only a proportion may reactivate, perhaps mainly those without anti-HBs, and surveillance of transplant units will continue to be required in any case for the early detection of primary infections.

The first of the patients described above and considered as a reactivation is still antigenaemic after eight years¹³ and carries on a useful working life with his renal graft in situ.

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NON-A, NON-B POST-TRANSFUSION HEPATITIS

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Summary To clarify the role of hepatitis-A virus (H.A.V.) in the aetiology of post-transfusion hepatitis unrelated to hepatitis-B virus, we have tested and titred pre-transfusion and convalescent serum samples from 32 patients for antibody to hepatitis-A antigen (anti-HA) by quantitative immune adherence haemagglutination. 12 patients had no detectable anti-HA in either pre-transfusion or late convalescent serum; the other 20 had anti-HA in pretransfusion serum and no significant change in titre during convalescence. This study excludes H.A.V. as the agent responsible for these cases of post-transfusion hepatitis and supports the existence of "non-A, non-B" hepatitis virus(es).

Introduction

SCREENING donor blood for hepatitis-B surface antigen (HBsAg) has helped to reduce the incidence of

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hepatitis after blood-transfusion.¹⁻³ Unfortunately, however, post-transfusion hepatitis has not been completely eliminated even though many blood-banks use sensitive radioimmunoassays to detect HBs Ag. Several seroepidemiological investigations have indicated that other agents besides hepatitis-B virus (H.B.V.) cause transfusion-associated hepatitis,^{1,4-6} and the fact that elimination of commercial donors reduces the incidence of both H.B.V.-related and H.B.V.-unrelated hepatitis supports this conclusion.^{1,3,7,8} Serological studies have shown that neither cytomegalovirus (C.M.V.) nor Epstein-Barr virus (E.B.V.) contributes significantly to post-transfusion hepatitis,^{3,5,6,9} but until recently, there was no serological test with which to evaluate the role of hepatitis-A virus (H.A.V.). In 1973, however, Feinstone et al.¹⁰ discovered by immune electron microscopy (I.E.M.) a virus-like particulate antigen, hepatitis-A antigen (HA Ag), in the acute-phase stools of patients with MS-1 strain H.A.V. infection. Using a stool filtrate containing HA Ag particles, these investigators demonstrated a serological response by I.E.M. in serum pairs from patients with type-A hepatitis but not type-B hepatitis or other illnesses. Applying this new technique to the evaluation of serum pairs from 22 patients with post-transfusion hepatitis, Feinstone et al. did not detect serological responses to HA Ag.¹¹ On the basis of these data, they concluded that H.A.V. plays little, if any role in the aetiology of transfusion-associated hepatitis, and Knodell et al., using the same I.E.M. technique, confirmed this finding.¹²

Although I.E.M. has been shown to be a reproducible,

TITRES OF ANTIBODY TO HEPATITIS-A ANTIGEN (ANTI-HA) IN PRE-TRANSFUSION AND CONVALESCENT SERUM FROM PATIENTS WITH TRANSFUSION-ASSOCIATED HEPATITIS

Patient	Anti-HA titre		Months after transfusion
	Pre-transfusion	Convalescent	
1	<1/10	<1/10	7
2	<1/10	<1/10	7
3	<1/10	<1/10	12
4	<1/10	<1/10	4
5	<1/10	<1/10	6
6	<1/10	<1/10	8
7	<1/10	<1/10	5
8	<1/10	<1/10	6
9	<1/10	<1/10	6
10	<1/10	<1/10	5
11	<1/10	<1/10	6
12	<1/10	<1/10	6
13	1/10	1/10	7
14	1/100	1/400	6
15	1/200	1/100	6
16	1/100	1/400	5
17	1/200	1/500	6
18	1/400	1/500	6
19	1/500	1/500	40
20	1/500	1/1000	7
21	1/1000	1/500	6
22	1/1000	1/2000	6
23	1/1000	1/2000	6
24	1/2000	1/1000	3
25	>1/1600	1/1000	6
26	1/1000	1/2000	6
27	1/2000	1/2000	6
28	1/2000	1/1000	6
29	1/2000	1/2000	6
30	1/2000	1/2000	6
31	1/4000	1/4000	18
32	1/8000	1/4000	6
	1/8000	1/8000	5
			6

sensitive, and quantitative method for detecting antibody to HA Ag (anti-HA),¹³ the ability to determine antibody quantitation by I.E.M. has been questioned.¹⁴ With the development of an immune adherence haemagglutination (I.A.H.A.) assay for anti-HA,¹⁵ however, questions of precision in antibody titration can be answered. Accordingly, to resolve questions about the role of H.A.V. in non-H.B.V. cases of transfusion-associated hepatitis, we have re-evaluated by I.A.H.A. the serum pairs from 32 patients, some of whom had been studied previously by I.E.M.

Materials and Methods

Patients

22 patients with transfusion-associated hepatitis unrelated to H.B.V. C.M.V., or E.B.V. were studied at the National Institutes of Health (N.I.H.), Bethesda, Maryland, and included in Feinstone's original report.¹¹ Since the original study a similar group of 10 patients has been followed at the N.I.H. Details of criteria for patient inclusion and schedules of serial serum collection have been described previously.^{11,16} The following serological methods were used to exclude HB virus, C.M.V., and E.B.V. infection: HBs Ag determination by solid-phase radioimmunoassay; antibody to HBs Ag by radioimmunoprecipitation or passive haemagglutination; antibody to hepatitis-B core antigen by a radioimmunoassay blocking test or by radioimmunoprecipitation; antibody to C.M.V. by complement fixation; and antibody to E.B.V. by immunofluorescence as described in previous reports.^{11,16} Clinically the original 22 patients and the new 10 are a homogeneous group, all of whom were multiply transfused during open-heart surgery with blood from volunteer donors negative for HBs Ag by radioimmunoassay. Incubation periods ranged from two to fifteen weeks between transfusion and onset of hepatitis.¹¹

Antibody to Hepatitis-A antigen (anti-HA)

Pre-transfusion and late convalescent serum pairs spaced at least three months apart were tested by I.A.H.A. as described by Miller et al.¹³ The unavailability of marmoset-liver-derived HA Ag prompted us to use HA Ag purified by isopyknic banding in caesium chloride and rate zonal separation in sucrose from stools of patients with acute type-A viral hepatitis, as described by Moritsugu et al.¹⁷ All samples were screened at dilutions of 1/10, 1/100, and 1/1000. End-point titrations were performed on serial two-fold dilutions of serum samples positive at screening dilutions.

Results

Anti-HA titres of pre-transfusion and convalescent serum samples for the 32 hepatitis patients are presented in the accompanying table. 12 patients had no detectable anti-HA in either pre-transfusion or convalescent samples. The other 20 had anti-HA in pre-transfusion samples; 18 had no significant change in anti-HA titre, but 2 patients (numbers 14 and 16) had a titre of 1/100 before transfusion and 1/400 six and five months later, respectively.

Discussion

These I.A.H.A. serological results are best understood when evaluated in comparison to the typical humoral immune response during H.A.V. infection. Studies in man and non-human primates indicate that the presence of anti-HA in pre-inoculation serum correlates with immunity to H.A.V.^{11,18,19} and no case of H.A.V. infection has ever been detected serologically in any subject with pre-existing anti-HA, regardless of size of infecting inoculum.²⁰ After inoculation, there is an incubation

period of two to six weeks, usually two to four, after which time serum-aminotransferase activity is raised and morphological changes of hepatitis occur in the liver. Anti-HA detectable by I.A.H.A. appears one to four weeks after acute illness, and titres rise gradually towards a peak in the range of $1/10^6$ to $1/10^5$ about two to three months after acute illness. Even though titres fall from this peak, serum-anti-HA remains raised for years after illness.^{13 15 17 21} Finally, animals previously infected with H.A.V., when rechallenged, develop no hepatitis but experience an early transient boost in anti-HA titre about one to two weeks after inoculation and rapid return to pre-challenge anti-HA titres several weeks later.²²

Thus, it is unlikely that H.A.V. caused hepatitis in any of these 32 patients. 12 did not develop anti-HA in convalescent serum, and the remaining 20 had anti-HA in their serum before transfusion, suggesting immunity to H.A.V. Of these 20, however, 2 had pre-transfusion titres of $1/100$ and convalescent titres (five to six months after transfusion) of $1/400$. Although this four-fold rise might be considered significant for many viral and bacterial infections, this feeble response is atypical of an infection in which rises in titre of more than a thousand-fold are the rule. Furthermore, pre-transfusion anti-HA, even at a titre of $1/100$, correlates positively with immunity. Finally, it is unlikely that this four-fold titre rise even represents H.A.V. exposure, first, because rechallenge is not associated with acute hepatitis, which these patients developed, and second, because after rechallenge anti-HA titres fall rapidly to pre-exposure levels. By five to six months, no boost in anti-HA titre would be apparent unless there were continuous exposure. Most likely, these slight rises are insignificant and represent normal variation in low titres with time.

These data extend and confirm previous investigations of the aetiological spectrum of post-transfusion hepatitis. Among patients in the United States who develop hepatitis following transfusion of volunteer donor blood prescreened by radioimmunoassay for HBs Ag, 80–90% cannot be due to H.A.V. or H.B.V. on serological and epidemiological grounds.^{3 6 11 12 16 23 24}

Besides these data, other evidence for non-A, non-B agent(s) has been accumulating. Non-A, non-B hepatitis has now been recognised in Britain,^{25 26} in Australia,²⁶ Japan,²⁶ Costa Rica,²⁷ and, possibly, Germany.²⁸ Not only does non-A, non-B hepatitis occur world-wide, but it is apparently spread by modes other than transfusion. Non-A, non-B cases of hepatitis have been seen in hæmodialysis patients,²⁵ renal-transplant recipients,²² family contacts of patients with non-A, non-B hepatitis,²⁷ health professionals exposed to hepatitis patients but who denied overt parenteral exposure (R. H. Purcell, unpublished data, J. W. Mosley, unpublished data), illicit drug abusers,²⁹ and institutionalised mentally retarded persons.²² Also several instances of chronic hepatitis have followed acute non-A, non-B hepatitis.²⁶

We feel there are adequate data to support the existence of non-A, non-B virus(es). The clinical and epidemiological exclusion of non-viral hepatotoxic factors in the cases we have studied, the brevity of viraemia and absence of a chronic carrier state for H.A.V., and the quantitative serological investigations presented here exclude H.A.V. as the agent responsible for non-B post-transfusion hepatitis and add to the growing evidence

for the existence of one or more non-A, non-B hepatitis viruses.

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B-CELL ALLOANTIGEN ASSOCIATED WITH CHRONIC MESANGIOCAPILLARY GLOMERULONEPHRITIS

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Summary Thirteen patients with chronic mesangiocapillary glomerulonephritis (M.C.G.) defined by light and immunofluorescent microscopy were examined for possible B-lymphocyte alloantigen associations by means of antisera from multiparous mothers of M.C.G. probands. Three such sera reacted

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