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Subject Interest
VIROLOGY/BLOOD TRANSFUSION

Non-A, non-B hepatitis markers in the west of Scotland*

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Using an immunoelectrophoresis test, antigens and antibodies have been identified in sporadic cases of non-A, non-B hepatitis in haemophiliacs, renal disease patients and post-transfusion patients. Although an antibody has been found in 5–10% of such cases it is not found commonly in blood donors. The antibody is transient, possibly suggestive of it being directly involved in acute non-A, non-B hepatitis.

Key words: hepatitis, viral, human; antibodies, viral.

Introduction

The Glasgow and West of Scotland Blood Transfusion Service, in conjunction with the Hepatitis Reference Laboratory at Ruchill Hospital, Glasgow, instituted in 1980 an investigation into non-A, non-B hepatitis in west Scotland. At that time evidence for the existence of non-A, non-B infectious agents in the region came from three main sources: (i) cases of post-transfusion hepatitis where hepatitis B virus could not be implicated; (ii) sporadic cases, known to be negative for hepatitis A and B viruses; and (iii) episodes of jaundice in haemophiliacs who already had existing antibody to hepatitis A and B viruses. It was thought possible that at least some of these cases could be due to non-A, non-B virus or viruses. Stored sera were available from many cases and initially a search was made for any unusual antigens or antibodies present, either during or after the hepatitis episode. This paper reports our findings and illustrates the problems encountered in searching for a marker of an unknown virus.

Materials and Methods

The first technique tried was immunodiffusion (ID). Weak lines were obtained using haemophiliac sera as a source of antibody but although specific, ID is known to be both insensitive and consuming in time and reagents, and we considered it unsuitable for large scale testing.

An immunoelectro-osmophoresis (IEOP) technique[1] had been introduced in 1970 for HBsAg and anti-HBs screening of blood donors in the west of Scotland, and this has also been used successfully for screening plasma for high levels of anti-tetanus[2] and anti-diphtheria antibodies. Using this technique the samples which gave weak lines in ID were further tested by IEOP, and after 1.5 h good strong lines were obtained showing that some specimens

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TABLE 1
Prevalence of antibody against FR-antigen in various population groups

Category	Number of samples tested	Number with antibody (%)
Renal unit patients	35	0 (0)
Hepatitis patients	404	66 (16.3)
History of hepatitis–jaundice	54	4 (7.4)
Alcoholic liver disease	67	1 (1.5)
Blood donors	1126	0 (0)

contained an antigen and others reacted against it. It was found possible to adapt the early system of screening for HBsAg and its antibody[1] to screen all specimens in the present investigation for antigen and antibody simultaneously.

Early in the testing programme it was realised that some of the lines produced were non-specific. In addition, IgG zoning appeared between the wells, sometimes masking weak (but true) precipitation lines. To overcome these problems various agarose preparations were tested. Indubiose,[3] Litex,[4] Miles[5] and Gibco[6] preparations all produced non-specific lines, but one bottle of BDH agarose[7] purchased some years before produced no IgG zoning and the precipitation lines appeared specific. This agarose was used for all the tests reported here.

Early in 1980 a large sample of serum was obtained from a patient (FR) who had developed post-transfusion hepatitis about 6 months earlier. Tests showed that none of hepatitis A, hepatitis B, cytomegalovirus (CMV), nor Epstein–Barr (EB) virus were involved. In the IEOP tests the serum of FR appeared to have an unusual antigen when tested against haemophiliac sera. The volume of serum available allowed extensive testing of population groups to discover the prevalence of antibody to the FR antigen.

Results

Prevalence of antibody to FR antigen

From 46 haemophiliacs 167 sera were examined for the presence of antibody against FR. Only 10 (16%) samples were found to have antibody. These positive samples were from four (9%) haemophiliacs, each of whom had three known episodes of jaundice. Three of the episodes were caused by acute hepatitis B infection but the remaining nine could not be correlated with any known infectious agent. The appearance of antibody in these patients appeared to coincide with an episode of jaundice (Fig. 1). Generally, antibody to FR antigen was not detected before jaundice, usually only during the episode of hepatitis, and normally became undetectable (in our system) on recovery of the patient.

A number of other population groups were tested against the antigen FR, and the results are shown in Table 1. Renal dialysis patients are receiving increasing numbers of blood transfusions, and in 35 such patients tested against FR antigen, no antibody was detected. Hepatitis is uncommon among renal patients in Glasgow but one episode occurred in a female patient in 1980. This patient was negative before the hepatitis episode but had antibody against FR during the hepatitis. Her antibody became undetectable shortly after her SGPT levels returned to normal, approximately 4 weeks after onset of jaundice.

A total of 404 specimens from patients with clinical evidence of hepatitis attributable to neither A or B virus were screened against FR antigen. From 53 different patients 66 specimens (16.3%) had demonstrable antibody. The patients ranged in age from 8 to 84 years, with a male/female ratio of 3:2. Sufficient serum was available from 55 specimens to allow SGPT tests. Over 60% of the patients with antibody had elevated SGPT levels, with 50% having over twice the upper normal limit and 8% over four times that limit.

A retrospective study was carried out on patients who gave a prior history of hepatitis or jaundice. Sera were available from such patients in 1978–79 which were known to be

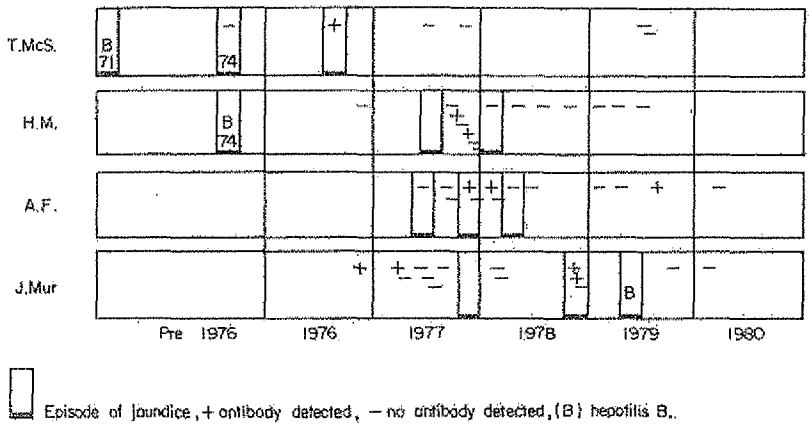

Fig. 1. Haemophiliacs tested for antibody. Hundred and twenty samples from 42 other haemophiliacs proved to be all negative.

negative for both anti-HBs and anti-HAV, and whose hepatitis episode was therefore unlikely to be a result of infection by A or B virus. Tests on 54 such sera showed only four (7.4%) to have antibody to FR antigen.

As the antibody to FR antigen appeared to be more common in liver disease patients a series of 67 patients with diagnosed alcoholic liver disease were screened. Only one weak positive result was obtained.

To find the prevalence of this antibody in the normal population, sera from 1126 blood donors from the west of Scotland were examined, and all were negative.

Prevalence of FR-type antigen

Several antibodies detected by the FR antigen were used to screen patients with liver problems for antigen of the FR type. Approximately 70% of these patients showed evidence of a circulating antigen, using the IEOP test system. Overall, a comparable prevalence of FR-type antigen was found in blood donors, varying from 32—100% depending on the antibody used (Table 2) — although it should be noted that many of the precipitin lines were very weak. Only one donor out of 516 tested for SGPT had a level over four times the upper limit of normal, and only seven (1.35%) had a level of more than twice the upper limit. The donor population used included 352 prison donors, among whom hepatitis B infection is much more common than among the ordinary blood donor population,[8] and among whom it was expected that markers of non-A, non-B hepatitis might also be more common (Table 3).

Stored specimens

The presence of FR-type antigen was readily detected in fresh blood donations or in fresh sera from patients with liver problems. Examination of blood donor sera stored for 6 months or longer at + 4 or − 20°C showed a dramatically reduced incidence. A similar low incidence was noted in other groups of sera stored for at least 6 months. Heat inactivation of fresh sera at 56°C for 30 min produced no reduction in the incidence of antigen in fresh sera or in the strength of the precipitin lines.

Multiplicity of antigen—antibody systems

An experiment was conducted in which the edge-to-edge distance between the wells in the IEOP system was increased in steps, from the normal distance of 5 to 25 mm. As the

TABLE 2
Prevalence of FR-type antigen in blood donors

Screening antibody	Number tested	Number with antigen (%)
J. Mür (Haem)	110	35 (32)
M.S. (Spor)	10	10 (100)
J. Mul (Spor)	500	295 (59)
J.C. (P.T.H.)	202	149 (73)
G.McL. (Spor)	139	104 (75)

Clinical category: *Haem*ophiliac; *P*ost Transfusion *H*epatitis; *Spor*adic.

TABLE 3
SGPT testing of blood donors

Category	Number tested	Number with levels > 35	> 42	> 125
Prison donors	352	8	6	1
Other donors	164	1	1	0
Total	516	9	7 (1.35%)	1 (0.19%)

Units are Sigma–Frankel (SF) units per ml.
100 SF U/ml = 48 i u/l.

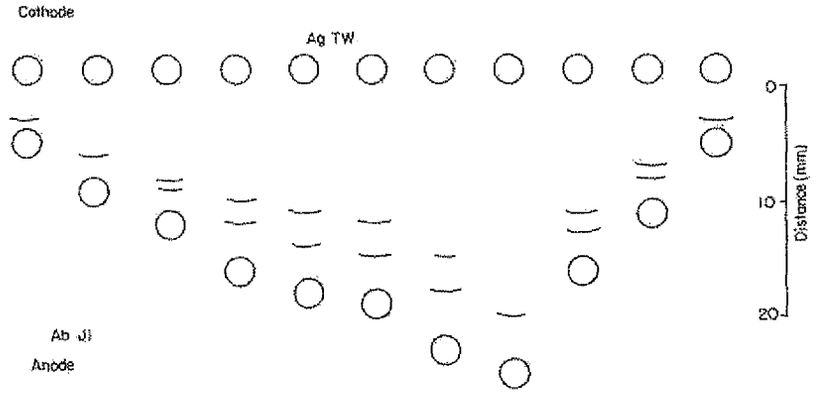

Fig. 2. Result of IEOP test in which sample TW (antigen) is tested against sample JI (antibody) whilst varying the distance of the antibody well from the antigen well.

separation increased, two different precipitin lines became clearly apparent in some tests (Fig. 2). It is likely, therefore, that neither the FR antigen – nor the antibodies detected against it – are monospecific, and that several antigen–antibody systems may exist.

Discussion

Results obtained so far from sera associated with unexplained hepatitis have demonstrated the presence of an unusual antibody in a proportion of such patients. This antibody is not present in normal blood donors, has appeared in the acute phase of hepatitis episodes in haemophiliacs and one renal patient, is present in 16.3% of patients with hepatitis, but is absent from patients with alcoholic liver disease. An antigen (or antigens) specific for the

antibody is apparently widely distributed in the population. Because of its distribution it is unlikely that this antigen–antibody system is associated with a non-A, non-B virus, although it does appear to be associated with some form of liver disease. Whether the antigen is indeed 'foreign', or whether the antibody is formed as a result of some auto-immune reaction, must await further investigation.

A hepatitis related antigen (HRA) and its corresponding antibody (anti-HRA) associated with non-A, non-B hepatitis have been reported by Neurath *et al.*[9] Of ten specimens containing antibody to FR antigen which have been examined by Dr Neurath, only two gave evidence of anti-HRA in a radio-immunoassay test, both patients having had prior transfusions. As our own evidence indicates that the antibody to FR antigen is polyspecific it is possible that some of our antibody-containing sera do contain anti-HRA as well as other antibodies. Hepatitis is a complex disease caused by a variety of agents, and often characterised by grossly abnormal serum immunoglobin levels. It is not surprising that a search for unusual antigens–antibodies in such a condition should reveal more than one new system.

The present work illustrates the many problems of searching for a new infectious agent in a disease as complex and diffuse as hepatitis. The index case of non-A, non-B hepatitis is usually in a haemophiliac, drug-abuser, or post-transfusion patient who may have developed jaundice as a result of a transfusion or a toxic reaction, and not from infection by an unknown agent. Only transmission experiments in chimpanzees can demonstate the presence of an infectious agent. Serological techniques can identify unusual antigens and antibodies, but only a small proportion of these may be markers of unusual infectious agents.

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