An Antigen Detected Frequently in Human Sera With Elevated Levels of Alanine Aminotransferase: A Potential Marker For Non-A, Non-B Hepatitis

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SUMMARY

In a search for additional antigens associated with virus-induced human liver disease a radioimmunoassay (RIA) was developed using IgG from sera of a multiply transfused person. Polystyrene beads coated with IgG F(ab')2 fragments, dinitrophenylated F(ab')2 fragments and 125I-labelled anti-2,4-dinitrophenyl antibodies (Neurath & Strick, 1979) were used in the RIA. An apparently new antigen or the corresponding antibodies were detected in 155 serum specimens from 35/37 (94%) individuals who developed non-A, non-B hepatitis. The antigen was also present in hepatitis B surface antigen-negative sera of blood donors with normal (13.2%) and elevated levels of alanine aminotransferase (34%). The antigen has an approximate mol. wt. of 45000, a buoyant density of 1.23 g/ml and an isoelectric point of 7.

INTRODUCTION

In a search for additional antigens associated with virus-induced human liver disease a radioimmunoassay (RIA) was developed using IgG from sera of a multiply transfused person. Polystyrene beads coated with IgG F(ab')2 fragments, dinitrophenylated F(ab')2 fragments and 125I-labelled anti-2,4-dinitrophenyl antibodies (Neurath & Strick, 1979) were used in the RIA. An apparently new antigen or the corresponding antibodies were detected in 155 serum specimens from 35/37 (94%) individuals who developed non-A, non-B hepatitis. The antigen was also present in hepatitis B surface antigen-negative sera of blood donors with normal (13.2%) and elevated levels of alanine aminotransferase (34%). The antigen has an approximate mol. wt. of 45000, a buoyant density of 1.23 g/ml and an isoelectric point of 7.

The use of sera from multiply transfused individuals for immunodiffusion studies on polymorphism of human serum proteins led to the discovery of Australia antigen and ultimately to the identification of hepatitis B virus (HBV; Blumberg, 1977). Attempts have been made to use such sera for the development of radioimmunoassays (RIA) for antigens associated with non-A, non-B hepatitis (Prince et al. 1978). Recent development of RIA tests in which amplified radioactive tracer binding is accomplished by the combined use of dinitrophenylated (DNP)-antibodies and 125I-labelled anti-DNP (Neurath & Strick, 1979), led us to search for antibodies reacting specifically with Dane particles (Alberti et al. 1978). In the course of these studies, we detected an additional antigen, distinct from all known antigens associated with hepatitis B. This antigen was present in a serum positive for both hepatitis B surface (HBsAg) and e-antigen (HBeAg). Subsequent tests revealed the presence of this antigen in a large proportion of sera from individuals who developed non-A, non-B hepatitis. The description of the RIA test, some properties of the antigen and its occurrence in sera from selected groups of individuals are the subject of this report. Because of its apparent relationship to hepatitis but a lack of direct evidence for an immunological relatedness to a virus causing non-A, non-B hepatitis, the described antigen will be designated temporarily as hepatitis-related antigen (HRA).
METHODS

Radioimmunoassay (RIA) for HRA. A recently developed RIA technique employing
\(^{125}\)I-labelled anti-2,4-dinitrophenyl (DNP) antibodies as a universal radioactive tracer
(Neurath & Strick, 1979) was used. Preliminary experiments in which IgG isolated from the
serum of a multiply transfused person (MS), which has a high titre of anti-HRA antibodies
(Fig. 3), was used for RIA, resulted in false positive tests with sera containing rheumatoid
factor. For this reason, F(ab)_2 fragments were used instead of IgG both for coating of poly-
styrene beads (performed as described before; Neurath et al. 1979) and for labelling with
2,4-dinitrobenzenesulphonate (sodium salt; Eastman Kodak Co., Rochester, New York).

IgG isolated from serum MS as described (Neurath et al. 1979) was incubated at 37 °C
overnight at pH 4.3 with pepsin (1 mg per 50 mg IgG). Solid tris(hydroxymethyl)amino-
methane (tris) was added to adjust the pH to 7, the mixture (4 ml) centrifuged at 63000 g
for 30 min and the supernatant solution mixed with 3 ml of a 10% suspension of Staphylo-
coccus aureus containing protein A (Pansorbin; Calbiochem-Behring Corp., La Jolla, Calif.)
to absorb undigested IgG. This was then clarified by low speed centrifugation. F(ab)_2 frag-
ments were separated from enzymic degradation products of pepsin and IgG Fe fragments
by gel filtration on a 2.6 × 19 cm column of Sephadex G-100 using 0.14 M-NaCl-0.01 M-tris,
pH 7.2 (TS), as eluant. Fractions containing the F(ab)_2 fragments were pooled, concentrated
to contain approx. 3 mg protein/ml and dialysed against 0.14 M-NaCl-0.01 M-phosphate,
pH 7.5 (PBS). To prepare DNP-labelled F(ab)_2 fragments, equal volumes of the dialysed
material and of 0.02 M-2,4-dinitrobenzenesulphonate in 0.25 M-NaHCO_3-Na_2CO_3, pH 9.5,
were mixed and incubated overnight, protected from light and subsequently dialysed
against TS.

To prepare immunochemically purified anti-DNP antibodies, 2 ml of a rabbit antiserum
to DNP-albumin (Miles Laboratories, Elkhart, Ind.) were chromatographed on a 1 ml
column of DNP-aminoethyl-cellulose pre-washed with 0.5 M-NaCl-0.02 M-phosphate,
pH 7.5 (PB). The column was washed with PB followed by 0.5 M-NaCl-0.2 M-carbonate
buffer, pH 10.9. Anti-DNP was quantitatively eluted by 8 M-urea-0.01 M-phosphate, pH 8,
and purified approx. 100-fold in this single-step procedure. Subsequently, anti-DNP was
extensively dialysed against 0.05 M-borate, pH 8.5, and labelled with \(^{125}\)I-Bolton–Hunter
reagent as described (Neurath et al. 1979). The labelled anti-DNP was diluted in 1% normal
rabbit serum (NRS) in TS to a radioactivity corresponding to 10000 ct/min/100 \(\mu\)l.

The DNP-aminoethyl-cellulose used for affinity chromatography was prepared by
reacting AE-cellulose (Sigma, St Louis, Mo.) with 0.05 M-2,4-dinitrobenzenesulphonate in
0.2 M-carbonate-bicarbonate buffer, pH 9.5, under conditions similar to those described by
Farah & Awdeh (1972).

For RIA tests, 75 to 400 \(\mu\)l of serum specimens were diluted with TS to a total volume of
400 \(\mu\)l and incubated overnight at 20 °C with polystyrene beads coated with F(ab)_2 frag-
ments isolated from serum MS. The beads were washed with TS, incubated for 2 h at 37 °C
with DNP-F(ab)_2 fragments (400 \(\mu\)l) prepared as described above and diluted 200-fold with
normal human serum (NHS) diluted 1:10 with foetal calf serum (FCS). Subsequently the
beads were washed with TS and incubated for 2 h at 37 °C with 400 \(\mu\)l of \(^{125}\)I-labelled anti-
DNP diluted fourfold with a mixture of FCS-NRS-NHS (4:5:4:5:1). Finally, the beads
were washed with TS and counted in a \(\gamma\)-counter. Results were expressed in ct/min or in
RIA ratio units corresponding to ct/min obtained for a specimen divided by ct/min
obtained for normal serum controls negative for HRA. Samples with an RIA ratio \(\geq 2.1\)
were considered positive.

To determine antibodies to HRA (anti-HRA), serial dilutions of specimens diluted in
300 \(\mu\)l of FCS (diluted 20-fold in TS) were mixed with 75 \(\mu\)l of an HRA-positive serum and
incubated for 30 min at 37 °C. Residual HRA was determined as described above.
Other methods. Molecular exclusion chromatography, isoelectric focusing, isopycnic gradient centrifugation, affinity chromatography on concanavalin-Sepharose, determination of hepatitis B surface (HBsAg) and e-antigens (HBeAg) and the corresponding antibodies (anti-HBs and anti-HBe) were all performed as described before (Neurath et al. 1978, 1979). Antibodies to hepatitis A virus (HAV) were determined with the HAVAB test kit from Abbott Laboratories (North Chicago, Ill.).

Serum alanine aminotransferase (ALT; synonymous with glutamic-pyruvic transaminase) was measured as described (Neurath et al. 1979). Levels $\geq 45$ International units (iu) were considered as elevated. Serum bilirubin was determined with commercial test kits from the American Monitor Corporation, Indianapolis, Ind. Levels $> 1.2 \text{ mg/dl}$ were considered as elevated. The criteria for infection by a non-A, non-B hepatitis virus were the following: two consecutive serum specimens with ALT values $> 45$ iu, with one of these at least twice as high as this level; no serological evidence of infection with hepatitis B virus (HBV), HAV or another viral agent known to cause hepatitis such as cytomegalovirus or Epstein-Barr virus and no other reasonable explanation for ALT elevations (Feinstein & Purcell, 1978; Stevens et al. 1978a).

Antisera to $\alpha$-fetoprotein, $\beta$-2-microglobulin and to liver-specific protein (LSP) were obtained from Accurate Chemical and Scientific Corporation, Hicksville, New York and Dr D. M. Jensen, Rush Medical Centre, Chicago, Ill., respectively.

Specimens containing HBsAg were centrifuged for 5 h at 420000 g in the SW 65 rotor (Beckman Instruments, Palo Alto, Calif.) to remove HBsAg. This was done because serum MS also contained antibodies to HBsAg. Further experiments revealed that this centrifugation step was not necessary. It was sufficient to add 40 $\mu$l of a solution containing 750 $\mu$g/ml each of HBsAg subtype $ad$ and $ay$ to the DNP-labelled F(ab)$_2$ fragments during the first incubation of the beads at $37^\circ\text{C}$ in order to inhibit the binding of F(ab)$_2$ fragments to HBsAg which possibly had become attached to the beads during the first incubation with the specimens at $20^\circ\text{C}$.

RESULTS

Detection of HRA in sera from selected groups of individuals

Using the RIA test described above, sera containing both HBsAg and HBeAg were screened for the presence of additional antigens. Positive results were obtained with some of the sera. These results remained unchanged after removing the major portion of HBsAg by centrifugation. HBeAg, purified as described (Neurath et al. 1979), was negative in the test. Rabbit antisera to HBsAg, Dane particles and the tubular forms of HBsAg, HBeAg and HBeAg, prepared as described before (Neurath et al. 1973, 1979), all failed to inhibit the test. To learn more about the properties of the antigen, one of the sera was chromatographed on Sephadex G-100 under conditions described for Fig. 4. Residual HBsAg was detected in fractions corresponding to the void volume of the column. HRA was detected in fractions no. 20 to 26 similarly as in Fig. 4. HBsAg was not detected in these fractions, thus excluding the possibility that HRA was a low mol. wt. breakdown product of HBsAg which would not have been removed by high speed centrifugation. In attempts to clarify the relationship between HRA and hepatitis B, additional sera positive for HBsAg as well as HBsAg-negative sera were analysed. The results are summarized in Table 1. Twenty six sera were from individuals pre-selected on the basis of very high HBsAg levels (positive in the AUSRIA test at dilutions $\geq 1:10^6$) and elevated ALT levels ranging from 51 to 208 iu. Eighteen of the sera (69 %) were positive for HRA. HRA detection was not related to ALT levels. Seventeen sera were from blood donors from Taiwan pre-selected on the basis of complement fixation titres of HBsAg $\geq 128$. Ten of the sera (59 %) were positive for HRA. To determine whether or not the frequency of HRA detection may be related to the selection of carriers with high
Table I. Detection of HRA in selected groups of individuals

<table>
<thead>
<tr>
<th>Characterization of group</th>
<th>Number positive for HBeAg</th>
<th>Number positive for HRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male homosexuals with high serum HBsAg titres and elevated ALT levels</td>
<td>26/26 (100 %)</td>
<td>18/26 (69 %)</td>
</tr>
<tr>
<td>Selected blood donors* with high serum HBsAg titres</td>
<td>17/17 (100 %)</td>
<td>10/17 (59 %)</td>
</tr>
<tr>
<td>Consecutive voluntary blood donors screened out as HBsAg-positive</td>
<td>9/35 (25·7 %)</td>
<td>2/35 (5·7 %)</td>
</tr>
<tr>
<td>HBsAg-negative voluntary blood donors† with normal ALT levels</td>
<td>—</td>
<td>8/61 (13·1 %)</td>
</tr>
<tr>
<td>HBsAg-negative voluntary blood donors† with elevated ALT levels</td>
<td>—</td>
<td>21/62 (33·9 %)</td>
</tr>
<tr>
<td>Haemophiliacs‡</td>
<td>—</td>
<td>15/50 (30 %)</td>
</tr>
<tr>
<td>Patients and staff members of haemodialysis units who developed non-A, non-B hepatitis</td>
<td>—</td>
<td>16/21 (76·5 %)</td>
</tr>
<tr>
<td>Cases of post-transfusion non-A, non-B hepatitis</td>
<td>—</td>
<td>[19/21: 90·5 %]§</td>
</tr>
</tbody>
</table>

* From Taiwan.  
† From New York.  
‡ 48/50 (96 %) were positive for antibodies to HBsAg and 17/50 (34 %) were positive for anti-HRA.  
§ Includes three cases with undetectable HRA in serum who developed anti-HRA.

levels of HBsAg, sera of 35 consecutive voluntary blood donors found to be positive for HBsAg were tested for HRA. Only 2 (5·7 %) were positive. Although HBeAg was detected by RIA in 9 (25·7 %) of the sera, none of these contained detectable HRA.

To establish whether the detection of HRA bears any relationship to hepatitis B or to liver disease indicated by elevations of ALT levels, sera of blood donors with elevated (62) and normal ALT levels (61) were tested for HRA. These sera were selected from a random sample of 13362 HBsAg-negative voluntary blood donors. There was no significant difference between the groups in either age (38 ± 12 years) or race. However, there were significantly fewer females in the group with ALT elevations (8·1 %) as compared with 30·6 % of the group with normal ALT; \( P < 0·01 \) in accordance with earlier data (Stevens et al. 1978b). HRA was detected in 21 (33·9 %) sera with an elevated ALT and only in 8 (13·1 %) of the control sera (\( P < 0·05 \) by the \( \chi^2 \) test with Yates' correction). Among the donor group with ALT elevations, differences in ALT levels between HRA-positive (range 45 to 225 iu; mean = 77 ± 43) and HRA-negative sera (range 45 to 112 iu; mean = 61 ± 17) were not statistically significant. The proportion of HRA-positive individuals in the group with or without ALT elevations did not appear to be sex-dependent.

In order to clarify the nature of HRA further, 171 serum samples of 36 individuals who developed non-A, non-B hepatitis were tested for HRA. Representative examples of the test results which are summarized in Table I are presented in Fig. 1. The ALT elevations were transient in seven cases (four patients undergoing haemodialysis and three cases of post-transfusion hepatitis; Fig. 1a) and coincided with the appearance of HRA. The ALT elevations were prolonged in 19 cases (seven patients undergoing haemodialysis, 11 cases of post-transfusion hepatitis and one homosexual male) and fluctuated, usually out of phase with HRA detection (Fig. 1, b and c). HRA remained detectable in some cases even after the return of ALT to normal levels. HRA was detected in single serum specimens from six out of seven additional individuals who developed non-A, non-B hepatitis. HRA was not detected in one haemodialysis staff member and two patients with transient, and one patient with chronic ALT elevations, presumably due to non-A, non-B hepatitis. However, two of
Marker for non-A, non-B hepatitis?

Fig. 1. Patterns of HRA and ALT levels in sera of two patients (b, c) and one staff member (a) of a haemodialysis unit with presumed non-A, non-B hepatitis. Top of vertical lines indicates HRA ratio units. Note differences between panels in scale for left ordinate. ●—●, ALT; ◯, titre of anti-HRA antibodies.

these HRA-negative individuals had antibodies to HRA (anti-HRA; see below). The peak ALT levels in the sera of the three HRA-negative cases were between 123 and 975 iu, suggesting that the appearance of HRA in serum is not merely due to liver damage as reflected in the increase in ALT level. HRA was detected in the serum from 15/50 haemophiliacs.

Bilirubin was determined in specimens from 21 individuals having elevated ALT levels in their serum. The concentration of bilirubin was elevated in sera from six individuals (28.6%), all of whom were positive for HRA. Normal levels of bilirubin were detected in specimens from ten HRA-positive and five HRA-negative cases.

Single HRA-positive specimens from 25 cases of non-A, non-B hepatitis were tested for HBeAg by RIA and were all negative.

Serum specimens from five homosexual males with transient ALT elevations, apparently
Fig. 2. Relationship between $^{125}\text{I}$-labelled anti-DNP bound to polystyrene beads and the dilution of HBsAg-negative serum from a blood donor with slightly elevated levels of SGPT (63 iu). A 1:20 dilution of foetal calf serum was used as diluent. Horizontal broken line indicates radioactivity corresponding to the diluent alone.

Fig. 3. Radioimmunoassay inhibition test for antibodies to HRA. Dose response curve for serum MS. Foetal calf serum diluted 1:20 with TS was used as diluent. Horizontal broken line indicates radioactivity corresponding to a positive control containing diluent and HRA.
Marker for non-A, non-B hepatitis?

Fig. 4. Gel filtration on a column (1.5 x 30 cm) of Sephadex G-100 of an HRA-positive and HBsAg-negative serum: ●—●, protein; ■■, results of RIA tests corrected by subtracting counts corresponding to diluent (1:20 FCS). The following proteins were used as markers for determination of mol. wt.: cytochrome c, ovalbumin, human serum albumin and IgG. Phenol red was used as marker for the included column volume.

due to infection with hepatitis A virus (HAV) (indicated by a rise of anti-HAV antibodies from undetectable levels before onset of hepatitis), were tested for HRA. HRA was not detected in the sera of two individuals, although peak ALT levels were about 1400 iu in each case. However, HRA appeared temporarily in specimens from three individuals at their peak ALT level (2500 to 4370 iu). One of the three individuals was simultaneously infected with HAV and HBV, as shown by transient appearance of HBsAg in his serum shortly after the time of ALT elevations. This indicates the occurrence of simultaneous infections with more than one hepatitis virus which might also involve non-A, non-B hepatitis virus(es).

One of the blood donors' sera containing HRA was selected for more detailed studies. Results in Fig. 2 indicate a non-linear relationship between the quantity of 125I-labelled anti-DNP attached to polystyrene beads and the level of HRA in serum. HRA was not detected when the serum was diluted more than 32-fold. Another selected serum obtained from a patient with acute non-A, non-B hepatitis was positive up to a 1000-fold dilution.

Relatively high titres of anti-HRA were present in serum MS which served as the source for the preparation of RIA reagents used in the test (Fig. 3). Similar levels of anti-HRA were detected in three distinct IgG pools prepared from the sera of multiply transfused haemophiliacs. The titres of anti-HRA in five distinct lots of normal immunoglobulin, obtained from various commercial sources, were much lower and corresponded to 50% inhibition endpoints at dilutions ranging between 1/10 and 1/320. Anti-HRA was detected transiently in two cases of non-A, non-B hepatitis with transient ALT elevations, either after clearance of HRA from serum (Fig. 1a) or at the time of elevated ALT levels in the serum of one staff member of a haemodialysis unit who remained HRA-negative. Anti-HRA was persistently detected in 2 of 19 patients undergoing haemodialysis. Seventeen of 50 haemophiliacs tested (34%) had anti-HRA in their serum.
Fig. 5. Isoelectric focusing of an HRA-positive serum (6 ml): •—•, protein; $\mathcal{S}$, relative concentration of HRA determined by RIA tests and calculated from a calibration curve (see Fig. 2).

Fig. 6. Results of isopycnic centrifugation in a CsCl gradient of HRA-positive serum: •—•, density; $\mathcal{S}$, results of RIA tests corrected by subtracting counts corresponding to diluent (1:20 FCS).
Marker for non-A, non-B hepatitis?

Some properties of HRA

Samples of seven distinct HRA-positive sera were chromatographed on Sephadex G-100. The results obtained with each serum were similar (Fig. 4) and revealed that HRA has an apparent mol. wt. of about 45,000. The isoelectric point of HRA determined by isoelectric focusing is approx. 7 (Fig. 5) and its buoyant density, estimated by isopycnic centrifugation in a CsCl gradient, is 1.23 g/ml (Fig. 6). HRA did not attach to concanavalin-Sepharose indicating the absence of carbohydrate residues with α-d-mannosyl groups. Extraction of serum with an equal volume of diethyl ether in the presence of Tween 80 (1 mg/ml) resulted in inactivation of HRA.

Since several antigens may appear in serum during liver disease: α-fetoprotein (Smith, 1971; Ruoslahti et al. 1974); the organ-specific but species-cross-reactive F-antigen (Smith & Iverson, 1973; Arakawa et al. 1976); possibly other liver-specific protein(s) (LSP; Dienstag, 1978); elevated levels of β2-microglobulin, probably in association with HLA antigens (Revillard et al., 1979), and carcinoembryonic antigen (CEA; Loewenstein & Zamchek, 1977), it was necessary to determine whether HRA was related to any of these antigens. Antisera to α-fetoprotein, LSP and β2-microglobulin were all negative in an RIA inhibition test (Fig. 3). Crude mouse F-antigen prepared as described (Smith & Iverson, 1973; Arakawa et al. 1976) was negative in RIA for HRA. Furthermore, HRA was destroyed by treating sera with papain (600 μg/ml) in the presence of 5 mM-cysteine for 2 h at 37 °C, while HLA antigens are released from cell membranes but not inactivated by treatment with papain (Trågårdh et al. 1979). The physicochemical properties of HRA are distinct from those of CEA (Loewenstein & Zamchek, 1977).

DISCUSSION

Approximately 10% of transfused patients become infected with hepatitis due to a virus(es) distinct from HAV, HBV or any other known virus (Aach et al. 1978; Alter et al. 1978; Tateda et al. 1979). The agent(s) has been designated as non-A, non-B hepatitis virus(es) and also appears to be involved in infections transmitted by means other than transfusion (summarized by Dienstag et al. 1979). In one study, the incidence of post-transfusion hepatitis was approx. ten times greater among recipients of blood with elevated ALT levels compared with donor blood having normal enzyme levels (Aach et al. 1978). The prevalence of a non-A, non-B hepatitis virus carrier state among blood donors may, perhaps, be even higher than data of post-transfusion hepatitis would suggest, since at least some recipients of blood would be expected to have antibodies to the corresponding virus(es).

Shirachi et al. (1978) recently detected a new antigen (designated hepatitis C) by immunodiffusion in acute-phase sera of 17 out of 23 patients with post-transfusion hepatitis type non-A, non-B. This antigen appears to have properties other than HRA described here. Antigens apparently related to non-A, non-B hepatitis were also detected by immunodiffusion in human sera (C. Trepo, personal communication) and by counter-electrophoresis in the sera of infected chimpanzees (R. J. Gerety, E. Tabor & M. Kabiri, personal communication).

Based on studies of post-transfusion hepatitis mentioned above, one would expect that at least 10% of apparently healthy blood donors would be carriers of non-A, non-B hepatitis virus(es) who, hopefully, could be identified by appropriate serological tests analogous to those used for screening of blood donors for HBsAg. Unlike Shirachi et al. (1978) who did not find ‘hepatitis C’ antigen in sera of healthy blood donors, we detected HRA in this group and the frequency of HRA detection increased among individuals with elevated levels of ALT. Anti-HRA was detected in only two of the non-A, non-B hepatitis cases who
had transient ALT elevations. Antibodies to 'hepatitis C' antigen were also detected transiently in a minor portion of cases which cleared 'hepatitis C' antigen (Shirachi et al. 1978).

The detection of HRA and the absence of anti-HRA in a considerable proportion of haemophiliacs (Table 1) and the appearance of HRA in serum of all cases of post-transfusion non-A, non-B hepatitis tested so far suggests that HRA is not an allo-antigen eliciting antibodies in individuals who are repeatedly transfused or exposed to plasma fractions.

HRA was present not only in sera of individuals apparently infected with a non-A, non-B hepatitis virus, but also in some persons infected with HAV or HBV. This finding could be ascribed to the high prevalence of HBV infections in the group of subjects available for our studies (patients and staff members of haemodialysis units and homosexual males) leading to occasional simultaneous or consecutive infections with more than one hepatitis virus. In fact, four out of the 37 non-A, non-B hepatitis cases had been previously infected by HBV since their sera were positive for anti-HBs. HBsAg-positive subjects would not be recognized as non-A, non-B cases since at present the diagnosis can only be made by exclusion in the absence of a specific marker for non-A, non-B virus(es). If HRA is indeed specifically related to a non-A, non-B hepatitis virus, the results presented here suggest frequent simultaneous infections with more than one hepatitis virus in selected populations.

The existence of more than one non-A, non-B hepatitis virus (Feinstone & Purcell, 1978) could partly explain why HRA was not detected in sera from all non-A, non-B hepatitis cases. The value of HRA as a potential marker for non-A, non-B hepatitis will have to be assessed in studies on blood transfusions involving analyses of sera from donors implicated in non-A, non-B post-transfusion hepatitis as well as from non-implicated donors.

We detected recently in the cytoplasm of several human embryonic and carcinoma tissue culture cell lines, an antigen indistinguishable from HRA (our unpublished data). These results suggest that HRA is a host antigen, of possible oncofoetal nature, the synthesis and release of which into serum is enhanced during non-A, non-B hepatitis and possibly during other infections. However, it cannot be excluded that the cell lines had been infected with a non-A, non-B hepatitis virus – a common human pathogen as discussed above.

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