Transplantation and Clinical Immunology

Volume X 1978

TRANSPLANTATION AND CLINICAL IMMUNOLOGY

VOLUME X

Proceedings of the Tenth International Course,
Lyon, May 22-24, 1978

This publication was made possible by a grant from the Fondation Mérieux

Editors:
J.L. Touraine
J. Traeger
H. Bétuel
J. Brochier
J.M. Dubernard
J.P. Revillard
R. Triau

1979

EXCERPTA MEDICA Amsterdam - Oxford

ISBN Excerpta Medica 90 219 0377 6
ISBN Elsevier North-Holland 0 444 90042 X
Library of Congress Catalog Card Number 76-478402 MARC

Publisher:
Excerpta Medica
305 Keizersgracht
1000 BC Amsterdam
P.O. Box 1126

Sole Distributors for the USA and Canada:
Elsevier North-Holland Inc.
52 Vanderbilt Avenue
New York, N.Y. 10017

Printed in The Netherlands by Groen, IJmuiden

Contents

Hepatitis viruses in transplantation and hemodialysis

Purine metabolism and lymphocytes

Artificial pancreas and pancreatic transplantation — technical and experimental aspects

Hepatitis viruses in transplantation and hemodialysis

IMMUNOTHERAPY OF HEPATITIS B

Arie J. Zuckerman

Department of Medical Microbiology and WHO Collaborating Centre for Reference and Research on Viral Hepatitis,
London School of Hygiene and Tropical Medicine,
London WC1E 7HT, England.

Much has been written during the last few years on all aspects of hepatitis B infection and an enormous bibliography has accumulated on the complex epidemiological, virological and immunopathological features of viral hepatitis (1, 2). In this paper three problems will be briefly considered: the isolation and propagation of hepatitis B virus in tissue culture, therapy with interferon and the safety of the experimental hepatitis B sub-unit vaccines.

TISSUE CULTURE

Attempts to isolate and propagate serially the hepatitis viruses have repeatedly failed (3, 4). The development of markers of infection with hepatitis B virus, the surface antigen and core antigen, have been studied by immunofluorescence and electron microscopy in primary embryo liver cell cultures, in cultivated fragments of liver biopsy and in embryo liver organ culture (3, 4, 5, 6, 7), but serial replication of a transmissible agent has not been demonstrated. The problem has now been investigated at a more basic level to determine whether, in the case of hepatitis B, the excess viral coat protein, represented by the surface antigen, could interfere with the adsorption of the virus by receptor blockage or by the induction of interferon (8). Interference assays were performed with representative RNA and DNA viruses, using whole sera from infected chimpanzees and purified surface antigen. Direct interference with the challenge viruses was studied by plaque and cytopathic effect reduction methods under a variety of conditions of antigen adsorption. Interferon induction was studied using the same test materials and, in addition, hepatitis B virus particles as inducers. Potentiation of interferon production was also attempted by priming and superinduction. Direct interference was not demonstrated and the surface antigen did not block the adsorption of other viruses to the cells (8). The serial propagation of hepatitis viruses in tissue culture remains an urgent problem with high priority. This step is essential for the complete characerisation of these agents, for studies on chemical and physical inactivation and for the development of conventional vaccines.

TREATMENT WITH INTERFERON

Interferons are small glycoproteins that are able to inhibit the replication of animal viruses. Two different interferons have been described from human cells: one produced in leucocytes and the other in fibroblasts. These interferons are distinguishable by differences in their physicochemical properties, antigenic structure and in their stability and host specificities.

Both leucocyte and fibroblast interferons are being used for the therapy of chronic hepatitis B infection, but details are so far available only for patients treated with leucocyte interferon. At Stanford University, Greenberg and associates (9) selected patients with chronic active hepatitis associated with hepatitis B infection on the basis of the following criteria: active disease for at least 6 months, abnormal liver function tests, histological evidence of piecemeal necrosis and circulating markers of hepatitis B virus. In all patients treated with daily injections of leucocyte interferon resulting in peak blood levels of 50-1250 units, DNA polymerase fell within 48 hours, followed by a fall in core antigen, $\bar{e}$ antigen and ultimately surface antigen in most patients. Treatment was continued as long as there was an improvement in these markers. In 3 out of 7 patients active infection was cleared, but in the remaining 4 markers of hepatitis B virus rebounded soon after treatment with interferon was discontinued.
At the University of London, Scullard and associates (10) treated 7 patients, selected by similar criteria, with 3 million units of leucocyte interferon injected intramuscularly daily for periods of 5 to 9 weeks. The virus associated DNA polymerase activity became negative in 3 patients and fell by a mean of 85% in another 2 patients in whom it was initially present. The number of complete hepatitis B virus particles in the serum, counted by electron microscopy, was also reduced and $\bar{e}$ antigen disappeared in 2 out of 3 patients during treatment. The effect, however, was not sustained beyond the first weeks of treatment. In addition, a marked decrease in lymphocyte-mediated cytotoxicity towards target cells coated with hepatitis B surface antigen was demonstrated.

It is clear that further studies are required based on careful selection of patients and attainment of adequate blood levels of interferon. There is some preliminary evidence that combination of interferon with adenine arabinoside may be effective for the treatment of patients who appear to be resistant to interferon alone.

THE ONCOGENIC POTENTIAL OF HEPATITIS B VACCINES

The safety of all viral vaccines especially in relation to oncogenicity must be carefully considered. This applies to possible contaminants of tissue cultures employed for the preparation of such vaccines, but in addition a number of viruses, particularly members of the herpesvirus group including herpes hominis type 2 and EB virus, have been implicated in the aetiology of certain human cancers. Such difficulties have been met, for example, in the

development by Elek and Stern (11) of a vaccine against mental retardation caused by cytomegalovirus (a member of the herpesvirus group) infection in utero, and the problem was discussed by McDougal and Harnden (12).

The geographical distribution of primary cancer of the liver presents intriguing epidemiological and demographic features. Numerous studies in many parts of the world, particularly in Africa, Asia, the Pacific and some Mediterranean areas, show a highly significant excess of hepatitis B surface antigen and core antibody in patients with primary liver cancer. These results may be interpreted in several ways. Hepatitis B is ubiquitous in areas where macronodular cirrhosis and primary liver cancer is common, and it is possible that patients with hepatocellular carcinoma are unduly susceptible to infection with hepatitis B and to the development of the persistent carrier state. However, it has been suggested that the important factor in the possible causal association between hepatitis B infection and liver cell cancer may lie in an early age of exposure to infection (13). Indeed, in areas of the world where the prevalence of macronodular cirrhosis and primary liver cancer is high, infection with hepatitis B virus and the carrier state occur most frequently in infants and children, and as many as 20% of the general population may be carriers. It appears likely, therefore, that persistent hepatitis B virus infection occurs before the onset of chronic liver damage. Another possibility is that persistent infection with hepatitis B virus leads to cirrhosis and that carcinoma then arises from regenerative nodules by mechanisms in which the virus is not involved. Such a mechanism may account for cases of liver cancer which occur in patients with alcoholic cirrhosis. This sequence, however, does not explain liver cancer associated with persistent hepatitis B infection in about 25% of patients in the absence of cirrhosis.

There is now additional evidence of a close association between hepatitis B and liver cancer. Macnab and her associates (14) derived a cell line producing hepatitis B surface antigen from a patient with primary liver cancer. Supernatant fluids from these cultures contain the surface antigen only and the antigen titre increases with time. The complete virus particles or the nucleocapsid of the virus have not been found in the cells nor in the supernatant fluid by a variety of sensitive techniques. This implies possible integration of part of the viral genome with the host cell genome resulting in production of excess viral coat protein. Hepatitis B virus contains a small circular double-stranded DNA and DNA polymerase activity. The endogenous DNA polymerase reaction uses the DNA as a template and therefore this reaction can be utilized as a specific probe by making radioactive virus particle DNA. Lutwick and Robinson (15) examined the effect of DNA extracted from the liver tissue of patients with persistent hepatitis B infection using the DNA product of the polymerase reaction. The amount of newly synthesised DNA was determined by the amount of nucleotide incorporation into an acid-insoluble form. DNA reassociation kinetics were used to determine the

complexity of the new DNA. The results suggested that a unique region or regions, corresponding to approximately 25 to 50% of the circular hepatitis B DNA template, was copied once during the reaction. DNA and RNA extracted from the livers of patients with persistent hepatitis B infection, chronic active hepatitis and hepatocellular carcinoma accelerated significantly the rate of reassociation of radioactive DNA from the virus. No enhancement was found when DNA extracted from uninfected healthy livers was used. The finding of virus alkali-stable DNA base sequences in rapidly sedimenting DNA molecules suggests that some of the sequences are probably attached or integrated into the host DNA molecules. Other studies indicated that viral DNA base sequences can be detected in RNA extracted from liver infected with hepatitis B virus but not in RNA from uninfected liver. These findings are consistent with transcription of viral DNA in infected liver cells.

The high rate of infection with hepatitis B virus in certain defined population groups in the developed countries and among the general population in many developing countries indicates the urgent need for a hepatitis B vaccine. The repeated failure to grow and passage hepatitis B virus serially in tissue culture has however hampered progress towards the development of a conventional vaccine. Since the separated 22 nm surface antigen particles of hepatitis B virus leads to the production of protective antibody, the possibility of using this material seems feasible. Several such experimental vaccines have now been prepared from the plasma of apparently healthy carriers of hepatitis B virus. Susceptible chimpanzees have so far been shown to be protected by such preparations treated with formalin and initial tests in volunteers of these experimental sub-unit vaccines are now in progress. However, before large scale clinical trials of the new unconventional hepatitis B yaccines are undertaken or serious consideration given to the suggestion of using these vaccines in children in the developing countries, it is essential to ensure that any benefit that accrues from immunization is not outweighed by a cancer hazard (16). Fortunately the necessary sophisticated technology to establish the complete inactivation of any live virus or residual DNA in such vaccines derived from potentially oncogenic viruses is now available.

REFERENCES

1. Zuckerman, A.J. (1975): Human Viral Hepatitis, 2nd Ed. p. 422. North Holland-American Elsevier, Amsterdam - New York.
2. Proceedings of a Symposium on Viral Hepatitis, National Academy of Sciences, Washington. (1975): Amer. J. Med. Sci., 270, 1.
3. Zuckerman, A.J. (1970): Virus Diseases of the Liver, p. 158. Butterworths, London.
4. Zuckerman, A.J. (1975). Amer. J. Med. Sci., 270, 197.
5. Brighton, W.D., Taylor, P.E. and Zuckerman, A.J. (1971): Nature new Biol., 232, 57.

6. Zuckerman, A.J. and Earl, P.M. (1973): Vox Sang. Suppl., 24, 123.
7. Watanabe, M., Umenai, T., Ohori, H. and Ishida, N. (1976): Brit. J. exp. Path., 57, 211.
8. Brumpt, I., Mann, G.F. and Zuckerman, A.J. (1978): J. med. Virol., in press.
9. Greenberg, H.B., Pollard, R.B., Lutwick, L.I., Gregory, P.B., Robinson, W.S., and Merigan, T.C. (1976): New Engl. J. Med., 295, 517.
10. Scullard, G.H., Alberti, A., Wansbrough-Jones, M.H., Howard, C.R., Eddleston, A.L.W.F., Zuckerman, A.J., Cantell, K., and Williams, R. (1978): Brit. med. J., in press.
11. Elek, S.D. and Stern, H. (1974): Lancet, 1, 1.
12. McDougal, J.K. and Harnden, D.G. (1974): Lancet, 1, 135.
13. Zuckerman, A.J. and Dunne, A. (1974): Gazz. Sanit., 23, 11.
14. Macnab, G.M., Alexander, J.J., Lecatsas, G., Bey, E.M. and Urbanowicz, J.M. (1976): Brit. J. Cancer, 34, 509.
15. Lutwick, L.I. and Robinson, W.S. (1977): J. Virol., 21, 96.
16. Zuckerman, A.J. (1978): Nature, 272, 579.

NON-A, NON-B HEPATITIS: REPRODUCTION OF DISEASE IN CHIMPANZEES AND IDENTIFICATION OF VIRUS SPECIFIC ANTIGEN AND ANTIBODY*

Alfred M. Prince, Betsy Brotman, M.C. van den Ende and Linda Richardson

Laboratory of Virology, The Lindsley F. Kimball Research Institute of The New York Blood Center, New York, U.S.A. and Vilab II, Liberian Institute of Biomedical Research, Robertsfield, Liberia

ABSTRACT

Hepatitis was reproduced by inoculation of filtrates of 11 specimens from 10 patients into 17 chimpanzees.

Liver tissue from one infected chimpanzee contained an antigen demonstrable by solid phase radioimmunoassay with antiserum derived from a polytransfused individual. This antigen appeared regularly in serum during the course of Non-A Non-B hepatitis in man and chimpanzee, and could be neutralized by antibody formed during the course of the disease. The antigen was not seen in cases of Type A or Type B viral hepatitis. This antigen persisted after its appearance in one human case for at least 6 months, suggesting the appearance of a carrier state, and the potential of this system for screening for carriers of a Non-A, Non-B hepatitis virus. This antigen and its antibody are tentatively termed Hepatitis Type C antigen (HC Ag) and anti-HC. The antigen has the characteristic density of lipoproteins (1.14 - 1.17 gm/cc).

In 1974 we reported that 36 of 51 cases of post-transfusion hepatitis occurring during the course of a prospective post-transfusion follow-up study did not show the serologic responses (HBsAg, anti-HBs, or anti-HBc) expected in infections with hepatitis B virus. (1) The long incubation period of the non B cases (8.0 ± 2.7 weeks), the lack of a protective effect of conventional γ globulin on their incidence, and the absence of secondary cases in the families of index cases, provided indirect evidence that these were not due to infection with hepatitis virus type A. This conclusion was soon strengthened by direct serologic evidence: 0/22 patients who developed non B post-transfusion hepatitis were found to have developed antibody to hepatitis A virus by immune electron microscopy in one study (2) and 0/34 in another (3).

The observation that the incidence of non B cases (rate per 1000 units transfused) was 10.2 times higher in recipients of paid vs. volunteer donor blood suggested, by analogy with the similar

*These studies were supported in part by a contract (AI62500) from the National Institute of Allergy and Infectious Diseases, N.I.H.; by General Research Support (BMRS) funds from the N.I.H.; by grant HL09011 from the National Heart, Lung and Blood Institute, N.I.H.; and by a Grant-in-Aid from Blood Services Inc., Scottsdale, Arizona.

findings in type B transmission, that this entity was a viral disease (4).

The possibility that either cytomegalovirus (CMV) or Epstein-Barr virus (EBV) may be involved in the etiology of Non-A/Non-B hepatitis has been considered. In the case of CMV most studies have found the incidence of CMV seroconversion to be indistinguishable in non B cases, B cases, and in persons not developing hepatitis (2), (5),(6). These findings do not support a role of CMV in the etiology on the non B cases. However, Knodell et al reported 8 fold or greater increases in the titer of CMV antibody in 3/30 Non-A/Non-B cases and in none of 20 randomly selected transfused controls who did not develop hepatitis (3). Furthermore, Luby et al noted a correlation in transfused patients (8). Thus a role for CMV as an etiologic agent in at least a proportion on Non-A/Non-B cases cannot yet be excluded.

Almost all transfused patients have EBV antibodies prior to transfusion (2),(3),(6). The presence of EBV antibodies appears to confer solid immunity to infectious mononucleosis (9),(10). It would thus appear a priori unlikely that EBV is responsible for this syndrome. Four fold or greater increases in antibody titer to EBV were not seen in any of 30 Non-A/Non-B cases by Knodell et al (3).

Due in part to the impact of sensitive methodology for detection of HBsAg carriers, the proportion of post-transfusion hepatitis cases due to non type A or B viruses appears to be rising. Two recent prospective post-transfusion follow-up studies have estimated the proportion to be 89% (11) and 88% (3).

Deinhardt and his colleagues isolated a hepatitis virus by inoculation of marmosets with acute phase serum from a human hepatitis case (12). This agent, now termed "GB" is clearly distinct immunologically and in physical properties, from both type A and B hepatitis viruses (13). Morphologically it appears to consist of fragile 20-22 nm particles identifiable in acute phase marmoset sera in the form of immune complexes (14). Although it has been suggested that the GB agent may represent a virus of marmoset origin (15) the fact that the original human serum specimen from which it was isolated produced hepatitis in each of 4 inoculated animals supports Deinhardt's interpretation that this may be a human hepatitis virus. If this is so, the GB strain must be considered an important candidate for at least one of the viruses of Non-A, Non-B hepatitis.

Another potential candidate agent is the parvovirus-like serum particle described by Cossart (16). This particle measures about 23 nm and has a density of 1.35 - 1.40 in CsCl resembling that of parvoviruses. The particles have been detected in the blood of healthy blood donors and also in acute serum from one patient with hepatitis. All follow-up sera tested from particle positive subjects revealed developments of precipitating antibody.

Attempts to isolate Non-A, Non-B hepatitis viruses in chimpanzees were initially unrewarding (Purcell R.H. Personal Communication; Barker L.F., Personal Communication). However recently we have succeeded in inducing Non-A, Non-B hepatitis in 17 chimpanzees with 11 "preacute" serum specimens from 10 human cases of this disease (17). Similar results have been obtained in three other laboratories (18),(19),(20). Herein we report identification of a virus specific antigen, tentatively termed HC Ag.

ISOLATION OF NON-A, NON-B VIRUS(ES) IN CHIMPANZEES

Since July 1977 thirty-three chimpanzees resident in our colony (Vilab II) at the Liberian Institute of Biomedical Research in Robertsfield, Liberia have been inoculated with 22 specimens taken from 17 patients with well documented Non-A, Non-B hepatitis. All isolation specimens were filtered through Millipore 0.45 and 0.22 micron filters. The inoculum volumes and dilutions are shown in Table 1. As summarized on this table, 11 specimens taken from 10 patients and inoculated into 17 chimpanzees produced hepatitis as defined by elevation of transaminase at least two standard deviations above the upper limit of normal for the individual chimpanzee being followed.

Six of the patients who provided infective inocula were cases of post-transfusion hepatitis occurring in our prospective post-transfusion follow-up study (4); one was a patient with dialysis-associated Non-A, Non-B hepatitis taken from our study of hepatitis B immune globulin as a preventive measure against dialysis-associated hepatitis (8); and four were Non-A, Non-B cases, three occurring in patients and one occurring in a staff member, taken from the ongoing surveillance of dialysis-associated hepatitis being undertaken by Szmuness and his colleagues. In all cases serum specimens for isolation were taken from the "pre-acute" stage of the illness, i.e., during the week before or the week of first elevation of transaminase.

Transaminase elevations in chimpanzees developing Non-A, Non-B hepatitis were usually mild, and frequently had a biphasic pattern. The highest transaminase (SGPT) observed was 340 Karmen units. SGPT always exceeded SGOT. In no case did the animals developing transaminase abnormalities develop HBsAg or anti-HBc, or alterations in anti-HBs, or anti-HA levels by radioimmunoassay.

All animals used in these experiments had pre-existing anti-HA and antiHBs levels resulting from prior exposure to HAV and HBV, or HBV vaccine.

Most animals were inoculated in pairs. Positive inocula uniformly resulted in induction of hepatitis in both members of an inoculated pair (Table 1); whereas negative inocula did not result in transaminase elevations in either member of inoculated pairs. From this we conclude that pre-existing immunity to Non-A, Non-B viruses in chimpanzees in our colony in Africa is rare or non-existent.

DEMONSTRATION OF VIRUS SPECIFIC ANTIGEN AND ANTIBODY

The removal, by partial hepatectomy, of approximately 50 grams of liver tissue from chimp 105 four weeks after onset of transaminase elevation (9 weeks after virus inoculation) permitted the development of a radioimmunoassay for a virus-associated antigen, and its corresponding antibody. For this purpose we employed a modification of the micro solid phase radioimmunoassay (Micro SPRIA) described by Purcell et al (22). Plates were coated with 1:10 dilutions of serum from four patients with hemophilia in 0.01 M Tris buffer, pH 9.0. After washing, the plates were soaked overnight at 4 C in their en-