ANNUAL REPORT

to the
Office of the Chief Scientist
of the
DEPARTMENT OF HEALTH AND SOCIAL SECURITY
ON THE WORK OF THE HEPATITIS LABORATORY

1983-1984

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

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1. Reference, training and advisory services

The hepatitis laboratory is designated as one of the NHS Hepatitis Radio-immunoassay Reference Centres for hepatitis B, and the laboratory also provides reference and diagnostic services for markers of infection with hepatitis A and hepatitis B viruses to a number of University Hospitals, District General Hospitals in the North-East Thames and South-West Thames Regional Health Authorities, and the North-East Thames Regional Blood Transfusion Centre, Brentwood.

The close links and collaborative work between this laboratory and have proved valuable and highly productive over a period of many years. Several joint research projects are in progress in addition to the provision of laboratory diagnostic services and more specialised examinations such as assay of DNA by hybridisation and electron microscopy.

There is an increasing demand for screening sera for total antibody to hepatitis A before administration of normal immunoglobulin for prophylaxis. This is a desirable development since it has resulted in a substantial reduction in the unnecessary use of immunoglobulin for individuals with hepatitis A antibody before travelling or working in areas of the world where hepatitis A is endemic.

Many requests for advice were received on policy, safety and indications for the use of the plasma-derived hepatitis B vaccine. Advice was sought by various official bodies, physicians and indeed members of the public.

The laboratory participated in the work of Advisory Committees of the Department of Health and Social Security and the Medical Research Council,
and in formal and informal consultations and meetings of the World Health Organisation.

Advisory services were provided to the Overseas Development Administration, the Foreign and Commonwealth Office, Regional and District Health Authorities of the NHS, the Zoological Society of London, the Pharmaceutical Industry and others.

The Department of Medical Microbiology and the Hepatitis Laboratory also houses the WHO Collaborating Centre for Reference and Research on Viral Hepatitis and as such it participates in various international reference, advisory and training activities.

2. Evaluation of reagents and standard preparations

The laboratory participates in collaborative national and international programmes of reagent testing and standardisation. A collaborative study is in progress with London, to assess the suitability of an alum adsorbed hepatitis B plasma-derived vaccine as an international (and national) reference preparation for immunogenicity. The proposed preparation would be used to determine the hepatitis B surface antigen content of vaccines prepared by different manufacturing procedures.

3. Research activities in viral hepatitis

3.1 Hepatitis B polypeptide micelle vaccine

The development of a polypeptide micelle vaccine has been described in detail in previous reports, and the work with micelles prepared from antigen derived from plasma was completed during the year. The safety, immunogenicity and efficacy of this type of preparation was established, and the results were confirmed by laboratories in the USA. The stage of
transfer of technology for industrial production was reached. Several problems were encountered during the familiarisation with the technique and its application at the Vaccine Production Laboratory of the Centre for Applied Microbiology and Research of the Public Health Laboratory Service, Porton Down. A decision was finally made to abandon the use of plasma in view of doubts concerning antigen derived from a source which may be contaminated with the agent of the acquired immune deficiency syndrome (AIDS), and the limitations on the availability of suitable plasma in the U.K.

It should be noted, however, that the micelle technique is being used in the U.S.A. for the preparation of micelles from a 22nm particle plasma-derived vaccine. Following safety and immunogenicity testing in chimpanzees, groups of healthy young adults have been immunised with the micelle vaccine (Hollinger F B, Sanchez Y, Troisi C, Dreesman G R and Melnick J L. Baylor College of Medicine, Houston, Texas, In press).

The micelle technique can be used for the preparation of vaccines from antigen sources other than plasma, for example from antigen expressed by recombinant DNA technology in yeast and in mammalian cells, and to antigen prepared by chemical synthesis. Much work was carried out with that aim in this laboratory as outlined below.

Standardisation of the content of hepatitis B surface antigen-specific polypeptides present in micelle preparations has been carefully examined using several different methods. Analysis of the final product by SDS-polyacrylamide gel electrophoresis followed by silver staining of the separated polypeptides allows a direct comparison of staining intensity with standard hepatitis B surface antigen (HBsAg) preparations of known concentrations.
Over 40 preparations of hepatitis B micelles have been reexamined to confirm (a) the purity of the final product (b) the consistency in the yields obtained, and (c) to obtain further results on antigenic quality by direct competitive radioimmunoassay. All results are consistent with the conclusions published previously by this laboratory.

The purification and inactivation procedures of the 22 nm HBsAg particles from the plasma of carriers were modified and additional inactivation steps were introduced because of concern that the starting material may contain the causative agent of AIDS.

Monoclonal antibodies and two synthetic peptides resembling amino acid sequences of HBsAg polypeptide I were used to define the specificity of antibodies induced in the mouse potency assay of the current and proposed revised WHO Requirements for plasma-derived hepatitis B vaccine (1981, 1983). Antibodies to the group specific determinant a of hepatitis B virus were shown to develop later, and the schedule for potency assay of vaccines has been revised and extended.

Independent confirmation of the work carried out at this School was published by Sanchez et al of Baylor University College of Medicine (Journal of Medical Virology, 1983, 11, 115-124). Increased immunogenicity and enhancement of surface antibody response by 20-40 fold were found with polypeptide micelles when compared to intact 2 nm surface antigen particles and SDS-denatured particles. The unaltered antigenicity of the micelles was demonstrated by substitution of intact surface antigen by 125I-labelled micelles as a detection probe for the surface antibody. Hepatitis B micelles have also been prepared by the same group from chemically-synthesized peptides (Sanchez Y et al. Intervirology, 1982, 18, 29-213) and aggregates,
possibly in micellar form, also by Hopp of the New York Blood Centre (Molecular Immunology, 1984, 21, 13-16).

The micelle procedure is being applied successfully in the Hepatitis Laboratory of this School to surface antigen particles expressed by recombinant DNA techniques in yeast (Saccharomyces cerevisiae). Preliminary results obtained in another laboratory (Burnette W N et al. In: Modern Approaches to Vaccines. Cold Spring Harbor Laboratory, New York, 1984, in press) confirmed the earlier findings of increased immunogenicity and enhancement of antibody response using the micellar technique. Further work is required, however, to define precisely the specific immunogenic peptides and to ensure purity of the solubilized immunopurified antigen and freedom from contaminating antigenic yeast material.

3.2 Molecular DNA hybridisation

The development of techniques which permit the detection of picogram amounts of hepatitis B viral DNA in liver biopsies and in serum permits the evaluation, at the molecular level, of the state of viral DNA during acute infection and in persistent infection.

Molecular characterisation of hepatitis B in chronic infection in the liver and serum of patients, including a group of patients treated with alpha-interferon is in progress (in collaboration with ...).

A dot hybridisation technique is being used to assay the levels of hepatitis B viral DNA and its relationship to serological markers of hepatitis in former blood donors who were found to be carriers on routine screening (in collaboration with Regional Blood Transfusion Centre, ...). Retrospective examination of serum samples
from carriers stored since about 1975 will be undertaken. This type of assay is also valuable for monitoring the response of patients to treatment with antiviral drugs, including interferon.

3.3 Non-A, non-B hepatitis

The development of specific serological tests for detection of markers of infection with the parenterally-transmitted forms of non-A, non-B hepatitis continues to elude laboratory workers in this field. Similarly the viruses have not yet been identified.

During the past year attempts were made in this laboratory to concentrate the infectious agent(s) from serum by ultracentrifugation. Transmission studies in London localised infectivity to the supernatant of documented infectious material and not in the pellet. This unexpected finding was confirmed by further studies in collaboration with the TNO Primate Centre in the Netherlands. Subsequent results, however, revealed infectivity in the middle of the gradient and it is evident that considerable further work will be required to localise and identify the infectious particles.

In another study, 114 liver biopsies obtained for diagnostic purposes from patients at Charing Cross Hospital were examined by transmission electron microscopy. The significance of intracytoplasmic crystalline structures found in the hepatocytes of 9 patients with various liver disorders was evaluated. The cytoplasmic inclusions (which have been described by others in hepatocytes of patients with acute post-transfusion non-A, non-B hepatitis and considered to be similar to those seen in infected chimpanzees) varied in size up to 2 µm in length and shape and were not limited by membranes. However, these inclusions, which might have served as diagnostic ultrastructural markers could not be correlated specifically with non-A, non-B
hepatitis, and the significance of these crystalline structures remains to be established.

There are reports in the literature that infection with hepatitis A in carriers of hepatitis B can result in resolution of the carrier state. This suggested a possible interference phenomenon. In an attempt to induce clearance of the carrier state, two carrier chimpanzees with very high titres of hepatitis B virus, were infused with non-A, non-B material. A substantial reduction in all markers of hepatitis B and viral DNA occurred, but the effect was transient. Similar findings were made with hepatitis A. Although disappointing, these results nevertheless indicate possible new approaches to the diagnosis of non-A, non-B hepatitis based on interference rather than on techniques dependent on antigen-antibody reactions. The development of specific laboratory tests for non-A, non-B hepatitis remains a matter of high priority.

Published work relating to this report:


