From the National Institutes of Health

Hepatitis Workshops of the National Institute of Allergy and Infectious Diseases

The Collaborative Research Program of the National Institute of Allergy and Infectious Diseases is sponsoring a series of workshops on hepatitis as part of its overall hepatitis program, which has as its goal the development of methods for the prevention of hepatitis A and B. Three recent workshops in this series include: (1) Antigenic Subtypes of Australia Antigen, December 17, 1971; (2) Hepatitis Animal Model Systems, January 14, 1972; and (3) Hepatitis and Immune Complexes, January 26-27, 1972.

Antigenic subtypes. The first of these three workshops focused on a discussion of the antigenic subtypes of hepatitis B. Hepatitis B antigen (HAA, HB Ag,1 or Australia antigen) was first detected in the serum of an Australian aborigine; later it was recognized that this antigen is associated with hepatitis B.

It is now known that sera from patients with hepatitis B may manifest several immunologic specificities as demonstrated by the double-diffusion precipitin reaction. At this meeting the participants agreed that an antigenic determinant known as \( a \) was common to all hepatitis B antigens thus far studied and may be regarded as the group-specific antigen. Either the \( d \) or the \( y \) determinant, but not both, is usually associated with the group-specific antigen \( a \). The \( D \) subtype, possessing both \( a \) and \( d \) determinants, accounts for 40%-50% of cases, while \( Y \) subtype, possessing both \( a \) and \( y \) determinants, also accounts for 40%-50% of cases. In less than 1% of blood samples tested, only the \( a \) antigen is detected. This might be because the subtype antigens are too weak for detection, are blocked, are destroyed, or are not made; or it might be because a new, unidentified subtype is present. Further research is needed to resolve the existence and character of other antigenic determinants. Some of the evidence presented suggests that the \( y \) determinant is more likely to occur in acute, self-limited hepatitis than is the \( d \) determinant. As a corollary, \( d \) is more likely to be found in chronic hepatitis and in the chronic asymptomatic carrier. This association between chronicity and the \( d \) subtypes suggests that persistent antigenemia relates at least in part to the infecting agent rather than being solely determined by response of the host.

It was reported that the 22-nm monomer forms of the \( ay \) and \( ad \) subtypes are composed of at least six polypeptides with varying amino-acid compositions. The participants of this workshop discussed nomenclature of subtypes and agreed that, until further information becomes available, the terms \( a \), \( d \), and \( y \) should continue to be used in describing antigenic determinants of hepatitis B.

Animal models. The workshop on hepatitis animal-model systems discussed methods of propagating the agents of hepatitis A and B. Past attempts to propagate hepatitis agents in tissue culture have been either unsuccessful or unsubstantiated. Recently, Carver and Seto [1] have exposed human WI-38 cells to human serum containing HAA; these cells are then rendered refractory to infection by Newcastle disease virus according to hemadsorption tests. This technique may represent one of the more promising approaches to growing the agent of hepatitis B in tissue culture. The use of nonhuman primates as animal models for hepatitis was discussed and analyzed as to its practicality. Participants in this workshop reported some success in infecting the chimpanzee and the rhesus monkey with hepatitis B. It was reported that general susceptibility of primates to hepatitis B, as recognized by development of antibody to the hepatitis B antigen, appears to parallel the phylogenetic scale, with those primates closest to man being the most susceptible. Studies on infection of primates with hepatitis A indicate that the marmoset can serve as a potential animal model for this type of hepatitis.

It appears at this time that certain nonhuman primates (e.g., chimpanzees, gibbons, orangutans) cannot serve as practical laboratory models for research on hepatitis, since these animals are a limited resource, very expensive, and diffi-

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1 The abbreviation HB Ag for hepatitis B antigen has been accepted by the Committee on Viral Hepatitis of the Division of Medical Sciences, National Academy of Sciences—National Research Council. However, pending editorial decision, The Journal retains the old abbreviation, HAA.
cult to handle and care for. The establishment of breeding colonies for such primates as *Saguinus mystax* and *Macaca mulatta* would be a great help in protecting the natural reservoir of these animals and in providing a source of good animals for research. As a consequence of the limited usefulness of certain nonhuman primates (chimpanzees, gibbons, etc.) in research on hepatitis, emphasis should be placed on development of tissue-culture techniques for growth of hepatitis agents and on development of potential materials for vaccines.

**Immune complexes.** The purposes of the workshop on immune complexes were to review current knowledge on immune complexes, to discuss the role that such antigen-antibody complexes might play on the pathogenesis of hepatitis B infection, and to attempt to formulate a hypothesis on the effects that the administration of gamma globulin with high titers of antibody to HAA would have on the formation of hepatitis immune complex.

Circulating antigen-antibody complexes are detected by a variety of procedures, including (1) use of isolated rheumatoid factors which precipitate aggregates of gamma globulin and immune complexes but not free gamma globulin; (2) use of Clq (an isolated component of complement) to precipitate immune complexes; (3) precipitation of immune complexes at low temperatures; and (4) use of the ultracentrifuge to separate complexes from free antigen and antibody. Other techniques that might be used with various degrees of success to detect hepatitis immune complexes include electrophoresis, complement fixation, immunofluorescence, platelet aggregation, and electron microscopy. In the future, radioimmunoassay might be applied to the detection of hepatitis immune complexes.

Immune complexes may cause the following reactions: (1) activation of complement; (2) degranulation of mast cells with release of such substances as histamine; and (3) release of platelet, polymorphonuclear, and lysosomal contents. The pathophysiologic consequences of these reactions could include contraction of smooth muscle, increased vascular permeability, destruction of tissue, and proliferation of endothelial cells. It was pointed out that the large immune complexes (greater than 19S) are most harmful, since they are not phagocytized and are deposited in tissue, causing the above-mentioned damage to that tissue.

The immune-complex syndrome might be summarized as follows: (1) formation of circulating antigen-antibody complexes; which cause (2) the release of vasoactive amines, followed by (3) increased vascular permeability leading to (4) deposit of the complexes along filtering vascular membranes, which causes (5) either nonpolymorphonuclear-mediated vascular injury or polymorphonuclear-mediated vascular injury through activation of complement. Deposition of immune complexes in the tissue might be due to deposition of circulating complexes or to deposit of antibody on cells in which viral replication takes place.

Current evidence supports the hypothesis that when the hepatitis B agent invades the body, two types of reactions are initiated. (1) The agent invades the liver cells, replicates, and antigen is released. (2) The hepatitis B agent stimulates the lymphoid system to produce antibodies. Because of the presence of both the hepatitis-B antigen and antibody, immune complexes are formed, which may either circulate or remain in local tissue sites.

Electron microscopic studies of liver biopsies from persons positive for the hepatitis B antigen indicate a variety of hepatic involvements. Immunohistochemical study of liver biopsies, using fluorescein-conjugated human antiserum to HAA or by indirect immunofluorescence with guinea-pig antiserum to HAA, demonstrates various patterns of distribution of this virus-associated antigen. The antigen is usually observed in the cytoplasm of hepatocytes in a focal distribution within the liver. It is less commonly found diffusely in the cytoplasm of hepatocytes. Granular deposition of the antigen in nonhepatocytes, including Kupffer cells, sinusoidal endothelial cells, and infiltrating mononuclear cells, is also seen, particularly in latter stages or during persistent infection. Evidence that immunoglobulin and complement components are deposited together in the last group suggests intrahepatic localization of immune complexes of antibody to HAA.

An electron microscopic study of three cases of hepatitis, representing the carrier state, chronic active hepatitis, and fulminant hepatitis, showed the following antigenic patterns, respectively: (1) persistent antigen and no evidence of anti-
body or complexes; (2) excess of antigen and presence of antigen-antibody complex; (3) excess of antibody and persistence of anaphylactogenic immune complexes. When there is free antigen, the patient is healthy, but with the development of antibody, hepatic function becomes abnormal.

Using the electron microscope, various investigators have seen Dane particles (42 nm), which are composed of an outer core and an inner, spherical (27-nm diameter) component. The outer core appears to be antigenically related to HAA, and the inner core bears some morphologic resemblance to the rhinovirus. The Dane particle can be disrupted with Tween 80 treatment into these two components, but the inner component may be unstable. Antibody to HAA and antibody to the inner core of the Dane particle might be associated with hepatitis B, although the role of these two antibodies in immune-complex formation or in protective immunity to hepatitis B infection is yet to be determined. Cell-mediated immune responses may also be involved in the conferral of immunity to hepatitis B. Cyclic adenosine 3',5' monophosphate and prostaglandins might also play a role in controlling the immune response to hepatitis B.

Based on different forms of evidence, the participants at the workshop agreed that hepatitis B disease may be complicated in certain cases by the formation of immune complexes. When these immune complexes lodge in the tissue, they initiate the typical immune-complex syndrome already outlined. It has been shown by various investigators that administration of certain gammaglobulin preparations can help prevent hepatitis B infection. It is not known whether the antibody to HAA or some other antibody, such as antibody to the inner core of the Dane particle, is the protective antibody. The effect of administration of gammaglobulin with high titer of antisera to HAA on immune-complex formation is not yet known. Particular caution should be exercised in administering gammaglobulin to persons positive for hepatitis-B antigen.

June Dunnick, Ph.D.

Infectious Disease Branch
National Institute of Allergy
and Infectious Diseases
National Institutes of Health
Bethesda, Maryland

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I. Carver, D. H., Seto, D. S. Y. Production of hemadsorption-negative areas by sera containing Australia antigen. Science 172:1265–1267, 1971.

Workshop on Coronaviruses

Research during the past few years has shown that members of the newly defined coronavirus group have many properties in common. Although coronaviruses cause illness in man, chickens, swine, mice, and rats, approaches to basic and comparative virology are seldom separable on the basis of species.

On January 31, 1972, the National Institute of Allergy and Infectious Diseases (NIAID) sponsored an international workshop to review the relevance and relationship of the presently known coronaviruses, to identify specific problem areas of mutual concern and interest, and to offer an opportunity for free exchange of knowledge. Nearly 20 investigators participated in the workshop, which was organized by the Collaborative Program of NIAID under the direction of Dr. Robert J. Byrne and was held at the National Institutes of Health, Bethesda, Maryland. Dr. B. C. Easterday, University of Wisconsin, Madison, served as chairman.

In a discussion of comparative methods and problems, Dr. M. Tajima of the Nippon Institute for Biological Sciences, Tokyo, described the fine structure of the nucleocapsid and the character of the nucleic acid of one of the coronaviruses, avian infectious bronchitis virus (IBV). He reported that, in his studies, most surface projections on IBV particles were lost during the process of purification. Many particles contained spherical corelike structures corresponding in size, shape, and location to amorphous material in two other coronaviruses, strain 29 E human coronavirus and the transmissible gastroenteritis virus of swine. Purified virus treated with lipid solvents was completely disrupted with release of the contents of the virus, but corelike structures could not be identified.

Dr. J. D. Almeida, electron microscopist at the Royal Post Graduate Medical School, London, England, described studies of antigen-antibody coronavirus reactions. Dr. Almeida reported that it is possible to distinguish virus and host antigens on the surface of avian IBV. How-