Hepatitis B

The Virus, the Disease, and the Vaccine

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CONTENTS

THE VACCINE

VACCINE ECONOMICS: ISSUES AND ANSWERS

VACCINES OF THE FUTURE

INTRODUCTION

Toby K. Eisenstein
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This symposium is the thirteenth biennial clinical microbiology program sponsored by the Eastern Pennsylvania Branch of the American Society for Microbiology in cooperation with the Philadelphia area medical schools and the Bureau of Laboratories of the Pennsylvania Department of Health. This year a generous contribution from Merck, Sharp and Dohme has helped to make the program a reality.

The subject matter for this symposium represents an attractive spectrum of medical, biological and molecular approaches to the practical solution of a public health problem--namely, prevention of infection with the hepatitis B virus. The symposium may be unique in that it focuses on a product which was first marketed less than three months ago, but included in the program are presentations on two new approaches to hepatitis B vaccine production which may replace the one which is newly unveiled. The rapidity of progress in our present era of biological research is indeed astonishing.

Vaccine development has been the major application, for human benefit, of research in microbiology and immunology. From Jenner's empirical observations in 1796 on protection of milkmaids against smallpox by cowpox infection, we have witnessed, through vaccination, the extinction in the 20th century of this ancient scourge of mankind (1). There are presently many novel and exciting approaches for improving existing vaccines, and for preparing new ones for organisms against which prophylaxis was never before available. The hepatitis B story illustrates how ingenuity in approach brought a solution to the problem of how to obtain an antigen which cannot be grown in tissue culture or laboratory animals.

In the case of the pneumococcus, Group B streptococcus, and Hemophilus influenzae, highly purified capsular polysaccharides are being used or developed as nontoxic vaccines (2,3,4). Biochemical coupling of antigenic determinants to carrier proteins, such as meningococcal polysaccharide to tetanus toxoid (5) and detoxified lipopolysaccharide of Pseudomonas aeruginosa to toxin A (6) are examples of molecular engineering applied to vaccine research. The isolation of the Texas Star strain of Vibrio cholerae, a mutant which produces the B or binding subunits of cholera toxin but not the A or toxic moiety, is an example of exquisite selection techniques to find the proper strain based on our appreciation of the molecular mechanisms of disease causation (7). For veterinary use, a chemically synthesized peptide vaccine has been produced against foot and mouth disease by sequencing the genome of the virus (8). In the last session of this symposium, we will hear about a synthetic hepatitis antigen, and also production of the hepatitis core antigen by cloning the gene in E. coli. Thus, vaccine development is currently an area of intensive investigation, where the newest methods are being applied to solve some of mankind's oldest problems.

For this symposium, we have gathered together researchers, clinicians and epidemiolgists to describe how each of these disciplines has contributed to the production of a vaccine against hepatitis B in the comparatively short span since discovery that the virus is the etiologic agent of the disease.

I know you will find the proceedings informative and exciting, so on behalf of the Eastern Pennsylvania Branch, I welcome you all.

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KEYNOTE ADDRESS: THE AUSTRALIA ANTIGEN STORY

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I would like to welcome visitors to Philadelphia and remind them that we are in the midst of celebrating our 300th birthday and looking forward to our fourth century as a thriving city. For the past year the city has undertaken an orgy of reminiscences and the commemoration of historic events. Philadelphia is gifted in preserving and recalling its past, and we would like to think that we are also interested in the development of an exciting future. All this historical reminiscing may provide an adequate excuse to look back at the work that we have done at the Institute for Cancer Research over the past 18 years which led to the discovery of the hepatitis B virus, the invention of the vaccine to protect against it, the possibility of prevention of primary cancer of the liver, and the many developments in our knowledge of this interesting virus. I hope that our extended discussion of the past won't detract from an interest in our current work, to which I will also refer briefly.

This work was accomplished by many investigators in our Institute. Figure 1 shows some of these. It is a photograph taken during September 1980 shortly after a site visit for one of our NIH program project grants. We have been fortunate in having an intelligent, dedicated and congenial group of scientists and staff working in our laboratory. It has been a great pleasure to be associated with them.

In this paper I plan to review our investigations beginning with the finding of Australia antigen and its identification as the surface antigen of hepatitis B virus. The narrative will proceed approximately chronologically, but themes will be

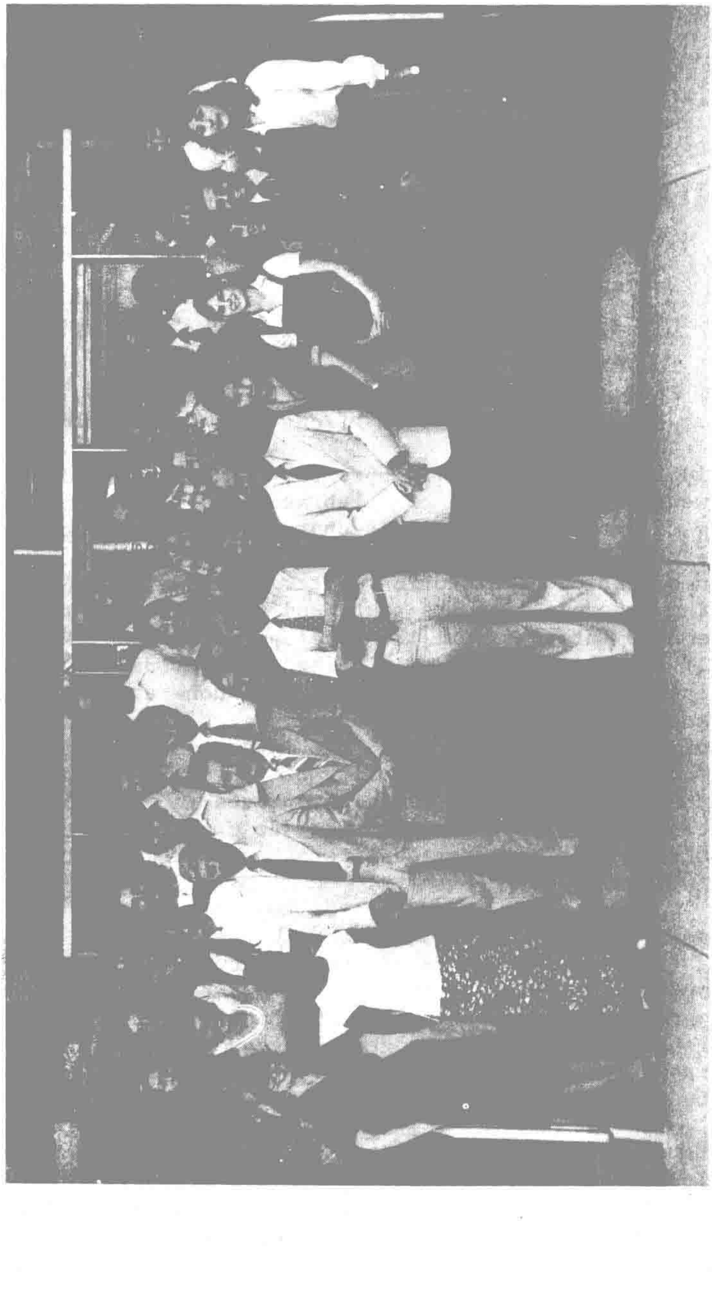

Figure 1. The staff of the Division of Clinical Research, Institute for Cancer Research, September 19, 1980.

developed out of linear time and into their eventual outcome. There will also be a digression to examine in detail how a scientific discovery, the identification of HBV carriers, became accepted into general medical and public health practice.

DISCOVERY OF AUSTRALIA ANTIGEN

In 1963 a major interest in our laboratory was the study of human biochemical and immunologic variation. A fundamental question that faces the physician is that of why some people become ill and others remain healthy even though all are exposed to the same disease hazard. Clearly, some of this is a consequence of chemical and immunologic variation in humans. We started in 1956 to study variation in serum proteins using the newly introduced starch gel electrophoresis method. We soon learned from studies in British, Basque, African, Alaskan and other populations that there was indeed a considerable polymorphic variation in several serum proteins (see, for example, references 1 and 2). We then made the hypothesis that if some of these serum protein variants were antigenic, transfused patients might develop detectable antibodies in their serum against variants which they had not inherited or acquired. We employed the method of double diffusion in agar gel using sera from transfused patients as the source of the putative antibody and testing these against other sera from normal people. Using this technique, we found a precipitating antibody that identified a complex inherited syster of serum low density lipoproteins which have since become of interest in genetic, anthropologic, forensic and other fields (3). The hypothesis of antigenic polymorphism had been supported by this observation, and we continued to test the hypothesis further by using sera from additional transfused patients to test against sera obtained from other populations. Since we were looking for unknown polymorphisms and the allele frequencies for most human polymorphisms vary greatly from population to population, we included in the serum panel against which the transfused sera were to be tested not only local populations but also those from Africa, Asia, Australia and elsewhere.

During the course of this ongoing research, a precipitin reaction dissimilar from any seen before was observed; and this reaction was between the serum from an Australian aborigine and that of a frequently transfused hemophilia patient from New York City (4). Figure 2 is an illustration, taken from an early publication illustrating such a precipitin reaction. (This is not the original Australia aborigine/hemophilia band, for which we do not apparently have a photograph.) What was this new phenomenon? What was the character and significance of "Australia antigen" (abbreviated Au), as we termed the protein present in the aborigine? In order to find out, it was necessary to formulate

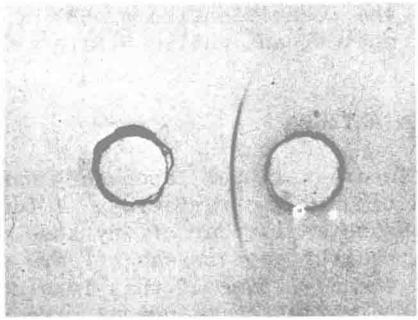

Figure 2. Precipitin reaction between serum from patient with "Australia antigen" (HBsAg) (top) and serum from hemophilia patient containing antibody against the antigen (anti-HBs) (bottom). Adapted from the first paper illustrating this reaction (4). (Reprinted by permission from JAMA 191:541-546, copyright 1965.)

a series of testable hypotheses, and additional observations were required to do this. Australia antigen was stable in sera kept in a frozen state, and we were able to test thousands of these taken from the large collection housed at the Division of Clinical Research of the Institute for Cancer Research. We learned that Au was very rare in U.S. populations but common (about 5-10%) in some African, Asian and Oceanic groups. We also learned that it was common in leukemia patients, most of whom had been transfused. Based on this observation, we made a series of hypotheses including the hypothesis that there is an inherited trait which makes people susceptible both to leukemia and to persistent carriage of the Australia antigen. To test this we generated a corollary hypothesis; namely, that people who have a high risk of developing leukemia should also have a high frequency of Au. Several such groups are known. Children with Down's syndrome (DS, mental retardation associated with trisomy 21) have a 20 fold or greater risk of developing leukemia. We tested groups of institutionalized DS patients and compared them to other mentally retarded children in the same institution (5). In all cases the frequency of Au was high in the DS patients ($\sim$ 30%) and much lower in the controls. This result was gratifying in that it not only fulfilled the predictions from the hypothesis, but also allowed us to observe a group of individuals who were closer to home than the Australian aborigines and other populations in whom a high frequency of Au was found. We learned that the presence or absence of Au appeared to be a persistent trait; if Au was present at first testing, then