Hepatitis B Virus Antigens in Tissues

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Foreword

Ever since the emergence of the concept of catharral jaundice by Virchow, viral hepatitis has eluded scientists as a pathogenetic enigma. A tremendous new impetus was given to hepatitis research by Baruch Blumberg's discovery of his 'Australia Antigen', now known as hepatitis B surface antigen. This led to an unheard-of outburst of research activity to elucidate the nature of the virus, its chemical and antigenic composition, its epidemiology, and pathogenetic mechanisms in the causation of liver disease. Coinciding with this period, modern medical science witnessed impressive progress in the analysis of the extraordinarily complex mechanism of immunological reactions. Immunohistochemical techniques for the detection of hepatitis B viral components are a product of this scientific progress in both areas. The application of such techniques forms the core of this work.

It represents a vast amount of work, performed during the course of several years, with meticulous application of advanced immunohistochemical techniques, combined with histopathology and clinical–pathological methods. This has resulted in the compilation of original results and new insights into the cellular and tissular localization of the antigenic components of the hepatitis B virus in different forms of chronic liver disease. The most outstanding results are the demonstration of the superior sensitivity of the applied immunohistochemical technique in the search for viral components in chronic hepatitis patients, and the differential distribution patterns of hepatitis B surface antigen in the various forms of chronic liver disease. Although the latter findings may not yet allow a complete understanding of viral replication and pathogenesis of liver cell damage in chronic hepatitis, it has been shown that they may serve as additional parameters in refining the diagnosis of the different forms of hepatitis B virus positive chronic liver disease.

In the last part of the work, an attempt is made to formulate a working hypothesis on the mechanism of liver cell necrosis, based on the original findings from the present work and on data from the literature. Even if the details of the presented hypothesis may have to be adjusted in the

light of future work, one feels that the basic framework will remain, involving complex interactions of both humoral and cellular immune reactions in the pathogenesis of hepatic and of extrahepatic tissue damage. More importantly, the findings described in this work, some of them already confirmed by other workers, will remain established facts not to be ignored by any future attempts to reformulate a working hypothesis on the pathogenesis of hepatitis B virus positive acute and chronic liver disease.

What Dr M. B. Ray has achieved is an important step forward in the understanding of the pathogenesis of chronic hepatitis, which still remains one of the most enigmatic challenges of today's medical practice.

Leuven April 1979 Professor Dr V. Desmet

Preface

The past decade has been marked by a tremendous advance in the histopathological and immunological aspects of hepatitis research. The central stimulus was the discovery of hepatitis B virus antigen (Australia Antigen) by Dr B. S. Blumberg in 1964. The articles, reviews and progress reports written on hepatitis B are innumerable; these are devoted mostly to epidemiology and serological studies. This monograph, however, gives exclusive attention to the behaviour of the virus in different kinds of tissues, to the process of viral replication in the liver, to the transport of the virus from the cell to the circulation, to the host-defence mechanisms against it and to the process of development of clinical hepatitis.

The text includes both the published and unpublished material included in a thesis submitted in 1978 to the University of Leuven for the degree of Ph.D by the author.

The book is divided into four main parts. Commencing with a short historical review on the discovery of the hepatitis B antigen and its association with diseases, the first part deals with a detailed description of the various methods for the demonstration of hepatitis B virus antigens in tissues, followed by their evaluation and subsequent applications in clinical medicine for assessing patients with hepatitis B and hepatocellular carcinoma. The second part describes the sequence of appearance of hepatitis B virus components and associated immunopathological changes in experimental hepatitis B. It also analyses the effect of interferon on the distribution patterns of the viral products in blood and liver. The third part presents an increasingly important subject: extrahepatic manifestations in hepatitis B virus infection. The fourth part reviews the present state of knowledge in the field of immunobiology along with a new approach to the understanding of tissue damage in hepatitis B virus infection.

I would like to acknowledge my indebtedness to my teacher, Professor Dr V. Desmet who initiated my interest in liver disease. The present work owes much to his unfailing encouragement, advice and indispensable support. I am grateful to Professor Dr J. Degroote and Professor Dr J. Desmyter for allowing me to study their patients, biopsies and sera.

Their help, advice and encouragement were invaluable to me. I specially thank Dr Fevery, Dr A. F. Bradburne and Dr Broekaert for their much needed cooperation.

I am most grateful to Professor R. N. M. MacSween, Department of Pathology, Western Infirmary, University of Glasgow, for his valuable remarks and suggestions in preparing this monograph.

I owe much to the help and cooperation of my colleagues and other staff members of this laboratory, in particular Dr C. Peeters, Dr B. van Damme and Dr R. de Vos. Special thanks are due to Dr R. de Vos for allowing me to use her electron microscopic data, and to Dr M. C. Kew, (Johannesburg) for the collaboration in the hepatocellular carcinoma work. It is indeed a pleasure to acknowledge Mrs M. Veulemans-Weckx, Mrs M. Vandenreyken-Bervoets and Mrs B. Tips-Smets for the huge amount of secretarial work needed in the preparation of this manuscript.

I thank Mr M. Rooseleers for his help in preparing the illustrations. I wish to thank the publishers and/or editors of the following journals for giving me permission to reproduce my published original material: *Journal of Immunological Methods* (Figures: 2.1A, 2.1B, 2.2A, 2.2B, 2.3, 2.4A, 2.4B); *Journal of Clinical Pathology* (Figure 3.3); *Gastroenterology* (Figures 2.11, 3.7, 4.1); *Clinical and Experimental Immunology* (Figures 5.1, 5.2, 5.3) and *The Lancet* (Chapter 8). Lastly, I deeply appreciate the support provided by the staff of the publisher, in particular Mr P. M. Lister, in preparing this monograph.

Leuven, April, 1979 M. B. Ray

Contents

Abbreviations

AHTC	acute hepatitis with signs of possible transition to chronicity
AMA	antimitochondrial antibodies
ANF	antinuclear factors
Anti-HBc	antibody to hepatitis B core antigen
Anti-HBe	antibody to hepatitis B e antigen
Anti-HBs	antibody to hepatitis B surface antigen
AusAb	radioimmunoassay kit (Abbott) containing materials for detection of antibody to hepatitis B surface antigen in blood
CAH	chronic aggressive hepatitis
CMI	cell mediated immunity
CPH	chronic persistent hepatitis
C-phase	core phase
DAB	diaminobenzidine
FITC	fluorescein isothiocynate
GAHu/C3/TRITC	goat immunoglobulin G against human complement component C3 conjugated with rhodamine
GAR/FITC/TRITC	goat antirabbit globulin conjugated with fluorescein or rhodamine
HBeAg	hepatitis B e antigen
HBV	hepatitis B virus
H & E	haematoxylin and eosin stain
HBsAg	hepatitis B surface antigen
HBcAg	hepatitis B core antigen
HCC	hepatocellular carcinoma
HLA	histocompatibility antigens
LMA	liver cell membrane auto-antibody
LSP	liver specific protein (antigen)

PAP	peroxidase–antiperoxidase complexes
PAS	periodic acid Schiff reaction
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
RAHu/C3/FITC	rabbit immunoglobulin G against human complement C3 conjugated with fluorescein
RAHu/IgG/FITC	rabbit antibody to human immunoglobulin G conjugated with fluoroescein
RIA	radioimmunoassay
SER	smooth endoplasmic reticulum
SMA	smooth muscle antibodies
S-phase	surface phase
SWAR/IgG	swine antibody to rabbit IgG
TRITC	tetramethyl rhodamine isothiocynate
VCF	*in vitro* complement fixation

General introduction – historical review

DISCOVERY OF AUSTRALIA ANTIGEN

The story of hepatitis B antigen began with an investigation of genetic differences in circulating low density lipoproteins. In the early 1960s, Dr B. S. Blumberg, a geneticist, began to evaluate the antigenic specificities of serum lipoprotein, which could be detected with sera of multiply transfused patients, e.g. in haemophiliacs who develop antibodies against certain lipoproteins (Blumberg *et al.*, 1962; Blumberg *et al.*, 1964). In

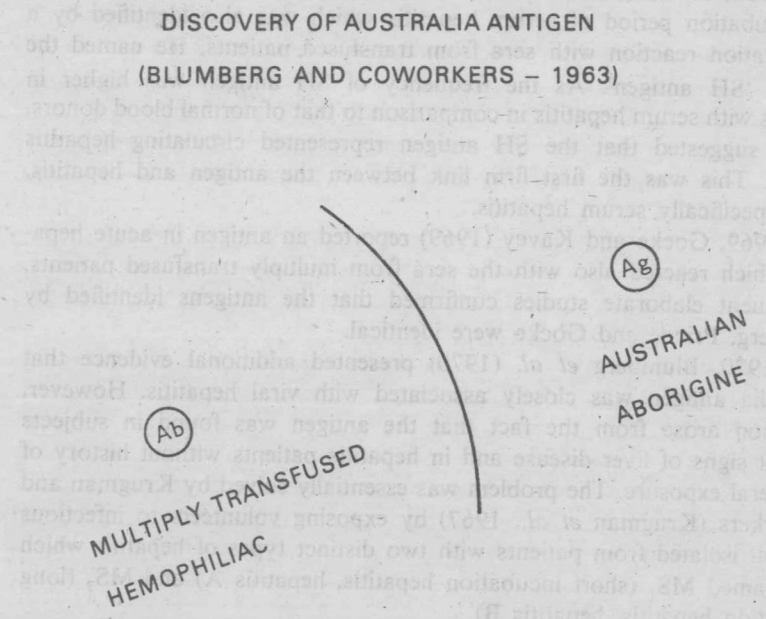

Figure 0.1 Diagramatic representation of Blumberg's discovery of hepatitis B antigen in Ouchterlony immunodiffusion agarose plate

1963, Blumberg (1964) tested sera from such patients for the presence of these antibodies by the Ouchterlony immunodiffusion technique. The panel of sera tested for the reaction with haemophilia sera included some obtained from foreign populations, one of which was from an Australian aborigine. This serum produced a precipitation reaction with sera from two haemophiliac patients which is shown diagramatically in Figure 0.1. Further study of the precipitin line showed that it differed from lipoproteins in that it stained only faintly with lipid stain but gave a strong reaction with protein stain. (Blumberg *et al.*, 1965). Although the antigen was later found not to be especially common in Australian aborigines, it was given the tentative name 'Australia antigen'.

ASSOCIATION WITH HEPATITIS B

The first association between Australia antigen and disease was the discovery of its high prevalence in patients with acute leukaemia but general absence or very low incidence in normal Americans and in patients with other diseases (Blumberg *et al.*, 1965). The antigen was later found to be sometimes present in patients with hepatitis (Blumberg *et al.*, 1967).

In 1968, Prince (1968) reported an antigen present in the blood during the incubation period of serum hepatitis which was also identified by a precipitation reaction with sera from transfused patients. He named the antigen 'SH antigen'. As the frequency of SH antigen was higher in patients with serum hepatitis in comparison to that of normal blood donors, Prince suggested that the SH antigen represented circulating hepatitis viruses. This was the first firm link between the antigen and hepatitis, more specifically serum hepatitis.

In 1969, Gocke and Kavey (1969) reported an antigen in acute hepatitis, which reacted also with the sera from multiply transfused patients. Subsequent elaborate studies confirmed that the antigens identified by Blumberg, Prince and Gocke were identical.

In 1970, Blumberg *et al.* (1970) presented additional evidence that Australia antigen was closely associated with viral hepatitis. However, confusion arose from the fact that the antigen was found in subjects without signs of liver disease and in hepatitis patients without history of parenteral exposure. The problem was essentially solved by Krugman and co-workers (Krugman *et al.*, 1967) by exposing volunteers to infectious material isolated from patients with two distinct types of hepatitis which they named MS_1 (short incubation hepatitis, hepatitis A) and MS_2 (long incubation hepatitis, hepatitis B).

Serum samples obtained during infection with MS_1 and MS_2 were examined for the presence of Australia antigen and SH antigen (Giles

et al., 1969). It was found that Australia antigen/SH antigen appeared exclusively in children infected with MS_2.

POLYMORPHISM OF HBV AND ASSOCIATED ANTIGENS

Viral structure and terminology

The nature of the hepatitis B antigens was determined by biophysical techniques and electron microscopy. The antigen can be purified by density gradient centrifugation (Gerin *et al.*, 1971). Electron microscopic examination of such purified material showed spherical particles of 22 nm in diameter and tubular forms sometimes over 100 nm in length. These particles do not contain nucleic acid. Subsequently, purified antigen obtained from patients with acute hepatitis was examined by Dane and co-workers (1970) and was found to contain an additional particle, 42 nm in diameter with a well defined coat on the surface and a core in the centre. This large particle is called the Dane particle and is generally thought to represent the hepatitis B virion (HBV). The structure and composition of HBV is schematically shown in Figure 0.2.

Antibody to Blumberg's Australia antigen reacts with the outer coat and not with the inner core of the Dane particle (Almeida *et al.*, 1971). A different antibody that reacts with the inner core has been found in the sera of patients with antigen positive hepatitis (Hoofnagle *et al.*, 1973).

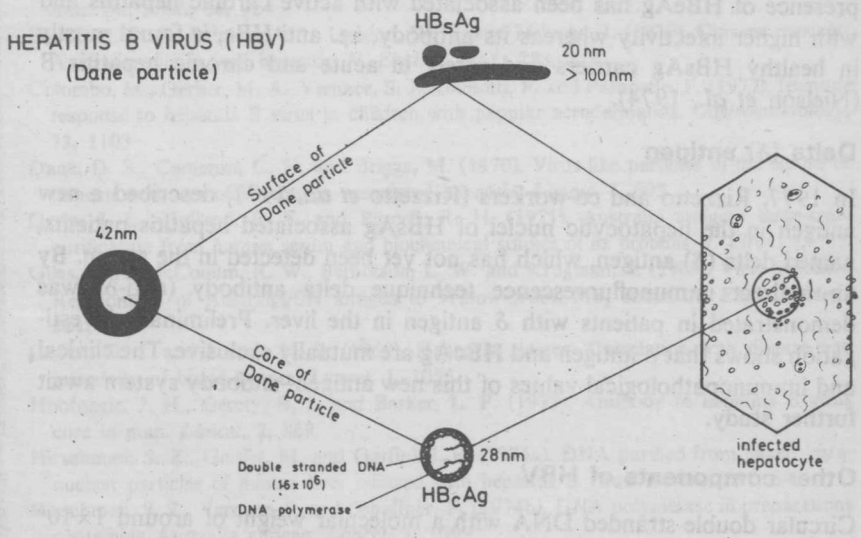

Figure 0.2 Schematic representation of the morphological forms of HBV antigens observed in blood and in hepatocytes

The identification of these two antigen systems led to a new nomenclature of the HBV antigens. The surface protein of the Dane particle and the major constituent of the spherical and tubular forms is termed hepatitis B surface antigen (HBsAg) and the core of the Dane particle is named hepatitis B core antigen (HBcAg). These two antigen systems can be identified by their respective antibodies, i.e. anti-HBs and anti-HBc.

Subtypes of HBsAg

HBsAg contains different subtypes, a group specific determinant a, and many additional mutually exclusive subdeterminant pairs, d–y and w–r. The w determinant also has mutually exclusive smaller subdeterminants. HBsAg positive sera can be divided mainly into four groups, adw, ayw, adr and ayr (Le Bouvier, 1972). These subtypes of HBsAg have been used as epidemiological markers but do not appear to have major clinical importance (Le Bouvier, 1973). Recently, however, infantile papular acrodermatitis has been associated with HBsAg subtype ayw (Ishimaru *et al.*, 1976; Colombo *et al.*, 1977).

'e' Antigen (HBeAg)

In 1972, Magnius and Espmark described a soluble antigen different from the classical subtypes of HBsAg in the sera of patients with HBsAg positive hepatitis and named it 'e' antigen (Magnius and Espmark, 1972). The presence of HBeAg has been associated with active chronic hepatitis and with higher infectivity whereas its antibody, i.e. anti-HBe, is found mostly in healthy HBsAg carriers and rarely in acute and chronic hepatitis B (Nielson *et al.*, 1974).

Delta (δ) antigen

In 1977, Rizzetto and co-workers (Rizzetto *et al.*, 1977) described a new antigen in the hepatocytic nuclei of HBsAg associated hepatitis patients, named delta (δ) antigen, which has not yet been detected in the serum. By an indirect immunofluorescence technique delta antibody (anti-δ) was demonstrated in patients with δ antigen in the liver. Preliminary investigation shows that δ antigen and HBcAg are mutually exclusive. The clinical, and immunopathological values of this new antigen–antibody system await further study.

Other components of HBV

Circular double-stranded DNA with a molecular weight of around 1×10^6 has been found in core particles isolated from infected human liver (Hirschman *et al.*, 1974a). The DNA isolated (mol. wt. 1.6×10^6) from

Dane particles is also found to be double-stranded (Overby *et al.*, 1975). A viral specific enzyme – DNA polymerase – has been identified in the Dane particle core (Kaplan *et al.*, 1973; Robinson and Greenman, 1974; Hirschman *et al.*, 1974b) and found to appear in the blood prior to clinical hepatitis (Krugman *et al.*, 1974). The exact site and mode of synthesis of viral DNA and DNA polymerase is still not clear. However, current evidence suggests that they may be synthesized in the hepatocytic cytoplasm (Hirschman, 1975). The details of viral replication are still obscure; hepatitis B virus seems to be an unusual type of DNA virus.

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