



STUDIES ON THE PATHOGENESIS OF  
HEPADNAVIRUS INFECTION

ALLISON RAE JILBERT

B.Sc (Hons)

Division of Medical Virology  
Institute of Medical and Veterinary Science  
Adelaide  
South Australia

Department of Microbiology and Immunology  
University of Adelaide  
Adelaide  
South Australia

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A thesis submitted to the University of Adelaide in fulfilment of the  
requirements for the degree of Doctor of Philosophy

January, 1989

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APPENDIX I - PUBLICATIONS ARISING FROM THE WORK REPORTED IN THIS THESIS

ABSTRACT OF Ph.D THESISSTUDIES ON THE PATHOGENESIS OF HEPADNAVIRUS INFECTIONALLISON R JILBERT

January 1989

Improved methods for the in situ hybridisation detection of messenger RNA (mRNA) in sections of liver tissue, were derived by use of an experimental system. This involved the use of tritiated-poly(dT) probes to detect poly(A) sequences attached to the 3' end of mRNA in sections of mouse liver that had been processed in various ways. The improved methods were applied to the detection of hepatitis B virus (HBV)- and hepatitis delta virus (HDV)-RNA. In situ hybridisation and immunostaining techniques were then applied to studies of the pathogenesis of HBV and duck hepatitis B virus (DHBV) infection.

In situ hybridisation studies of liver biopsy tissue from HBV-infected immunosuppressed renal transplant patients demonstrated an anatomical association between piecemeal necrosis and HBV replication at the cellular level in some patients. However, widespread replicative infection of hepatocytes also occurred in some patients in the presence of normal hepatocyte morphology and mild inflammatory changes indicating that at the cellular level virus replication was not necessarily a direct cytopathic process. These findings supported the view that hepatocyte injury may: (i) result from immune-mediated damage directed against cells undergoing replicative, but not restricted infection; (ii) eliminate cells undergoing replicative infection and favour clonal regeneration of cells undergoing restricted infection.

Localisation of interferon- $\alpha$  (IFN- $\alpha$ ) expression in liver tissue chronically infected with HBV and HDV, identified mononuclear cells and fibroblasts (but not hepatocytes) as the main producers of IFN- $\alpha$ . IFN- $\alpha$ -positive cells were associated with areas of liver tissue containing cells supporting virus replication and exhibiting the greatest degree of liver damage, suggesting that locally produced IFN- $\alpha$  may be a natural regulator of virus replication in chronic liver disease.

Experimental DHBV infection of Pekin-Aylesbury ducks showed that virus inoculated either intravenously or intraperitoneally, gained access to randomly distributed hepatocytes without first replicating in other cell types in the liver. Virus was seen to disseminate to contiguous cells following anatomical boundaries by the third day post-inoculation. Markers of DHBV infection in liver and serum showed reproducible kinetics, and duck hepatocytes in this system appeared to be highly permissive as large amounts of DHBV DNA and DHBsAg were produced intracellularly without the development of ongoing cytopathology. Hepatocytes were the major cell type responsible for early significant DHBV replication, in contrast to pancreas, kidney, spleen and circulating mononuclear cells where significant levels of infection were detected only after the first week of infection and the onset of viraemia.



## ACKNOWLEDGEMENTS

The work reported in this thesis has been performed in the Division of Medical Virology, Institute of Medical and Veterinary Science, Adelaide. I am indebted both to the IMVS and to the National Health and Medical Research Council of Australia for allowing me to enrol as a part-time post-graduate student in the Microbiology and Immunology Department of University of Adelaide.

I am deeply grateful to Professor Christopher J Burrell for his support, guidance and friendship throughout my Ph.D candidature. Dr Eric J Gowans has also contributed enormously to the success of my research work by providing excellent advice and a stimulating environment in which to study. My thanks must also go to Professor Barrie Marmion for his insightful appraisals of the work.

In addition, a large number of people and organisations have contributed to this work including:

My fellow students, Tom Macnaughton and Qiao Ming for their help and advice; Mr Angelo Izzo for determination of the autoradiographic efficiency of tritium in methacrylate resin; Dr Justin LaBrooy, Dr Michael Lawson, Dr Franco Negro and Dr John Gerin for provision of serum and liver samples; Professor Ken Murray, Dr B Baroudy and Dr John Gerin for HBV DNA- and HDV cDNA-containing plasmids; Dr Pauline Hall and Dr Robert Rowland who provided valuable histological advice; Amersham International for gifts of [<sup>125</sup>I]rCTP; Dr Paul Hertzog and Professor Anthony Linnane with whom I collaborated to study interferon- $\alpha$  expression in HBV-infected liver tissue; the Red Cross Blood Transfusion Service for gifts of buffy-coat leucocytes;

Dr K Cantell for polyclonal rabbit anti-interferon- $\alpha$ ; Dr John Freiman and Professor Yvonne Cossart who initiated my involvement in study of the DHBV animal model and supplied DHBV-positive serum; Tegel Duck Hatcheries who generously donated day-old ducklings; Dr Pat Marion and Dr John Pugh for gifts of DHBV DNA-containing plasmids; Members of the Photographic Services, IMVS, for their tireless assistance; Members of the Division of Tissue Pathology, IMVS, for provision of wax-embedded sections; and Dawn Campbell, Julie Maylin and Nina Wujda for excellent secretarial assistance.

Finally, my thanks must go to my friend Ian and to my family for their love and support during the period of enrolment for this degree.

ABBREVIATIONS

AA	amino acids
anti-HBc	antibody to hepatitis B core antigen
anti-HBe	antibody to hepatitis B e antigen
anti-HBs	antibody to hepatitis B surface antigen
anti-HBx	antibody to hepatitis B x antigen
AR	analytical reagent grade
bp	base pair
BSA	bovine serum albumin
CAH	chronic active hepatitis
CPE	cytopathic effect
CPH	chronic persistent hepatitis
Da	Dalton
dATP	2'-deoxy-adenosine-5'-triphosphate
dCTP	2'-deoxy-cytidine-5'-triphosphate
DDW	deionised distilled water
dGTP	2'-deoxy-guanosine-5'-triphosphate
DNA	deoxyribonucleic acid
dNTP	2'-deoxy-nucleotide-5'-triphosphate
DNase I	deoxyribonuclease I
ds DNA	double-stranded DNA
EAA	ethanol:acetic acid
eg	for example
dTTP	2'-deoxy-thymidine-5'-triphosphate
EM	electron microscopy
G	gravity
FITC	fluorescein isothiocyanate
H & E	haematoxylin and eosin
HCC	hepatocellular carcinoma
IF	immunofluorescence
IFN	interferon
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	intraperitoneal
IV	intravenous
mRNA	messenger RNA
MW	molecular weight

NDS	normal duck serum
NHL	normal liver homogenate
NHS	normal human serum
NRS	normal rabbit serum
OD <sub>260</sub>	optical density at 260nm
OD <sub>280</sub>	optical density at 280nm
OD <sub>260/280</sub>	ratio of optical density at 260 and 280nm
PBL	peripheral blood leucocytes
PBS	150mM NaCl, 7mM Na <sub>2</sub> HPO <sub>4</sub> , 3mM NaH <sub>2</sub> PO <sub>4</sub> . H <sub>2</sub> O pH7.2
PML	polymorphonuclear leucocytes
POPOP	1, 4-bis[2-(5-Phenyloxaxoly1)] benzene
PPO	2, 5-diphenyloxazole
PVP	polyvinylpyrrolidone
rATP	2'-ribose-adenosine-5'-triphosphate
rCTP	2'-ribose-cytidine-5'-triphosphate
RE	restriction endonuclease
rGTP	2'-ribose-guanosine-5'-triphosphate
RNA	ribonucleic acid
RNase A	ribonuclease A
RT	room temperature
rUTP	2'-ribose-uridine-5'-triphosphate
ss DNA/RNA	single-stranded DNA/RNA
SSC	0.15M NaCl, 0.15m Na <sub>3</sub> citrate pH7.0
TCA	trichloroacetic acid
TCID	tissue culture infectious dose
tRNA	transfer ribonucleic acid
uv	ultraviolet

All other abbreviations are as listed in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" by the International Committee of Medical Journal Editors (Annals Internal Medicine 1982; 96: 766-771).

PUBLICATIONS ARISING FROM THE WORK REPORTED IN THIS THESIS

(COPIES OF ORIGINAL PAPERS LOCATED IN APPENDIX I)

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