STUDIES ON THE PATHOGENESIS OF HEPADNAVIRUS INFECTION

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ABSTRACT OF Ph.D THESIS

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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-α (IFN-α) expression in liver tissue chronically infected with HBV and HDV, identified mononuclear cells and fibroblasts (but not hepatocytes) as the main producers of IFN-α. IFN-α-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-α 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.

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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 isothiocyante
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_{260}	optical density at 260nm
OD_{280}	optical density at 280nm
$OD_{260/280}$	ratio of optical density at 260 and 280nm
PBL	peripheral blood leucocytes
PBS	150mM NaCl, 7mm Na_2HPO_4, 3mM NaH_2PO_4. H_2O pH7.2
PML	polymorphonuclear leucocytes
POPOP	1, 4-bis[2-(5-Phenyloxaxolyl)] 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_3citrate pH7.0
TCA	trichloracetic 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)

1. **JILBERT AR, BURRELL CJ, GOWANS EJ, ROWLAND R (1986).** Histological aspects of *in situ* hybridisation: detection of poly(A) nucleotide sequences in mouse liver sections as a model system. **Histochemistry 85: 505-514.**

2. **JILBERT AR, HERTZOG PJ, BURRELL CJ, GOWANS EJ, LINNANE AW, MARMION BP (1986).** Production of a monoclonal antibody to human interferon-α (IFN-α) and its use to identify IFN-α-producing cells in virus infection *in vivo*. **Microbial Pathogenesis 1: 159-168.**

3. **JILBERT AR, BURRELL CJ, GOWANS EJ, HERTZOG PJ, LINNANE AW, MARMION BP (1986).** Cellular localisation of α-interferon in hepatitis B virus-infected liver tissue. **Hepatology 6: 957-961.**

4. **JILBERT AR, FREIMAN JS, GOWANS EJ, HOLMES M, COSSART YE, BURRELL CJ (1987).** Duck hepatitis B virus DNA in liver, spleen and pancreas: analysis by *in situ* and Southern-blot hybridisation. **Virology 158: 330-338.**

5. **JILBERT AR, FREIMAN JS, BURRELL CJ, HOLMES M, GOWANS EJ, ROWLAND R, HALL P, COSSART YE (1987).** Early virus-cell interactions in duck hepatitis B virus infection: a time-course study of tissue tropism and sites of virus production. **In: Zuckerman AJ, ed. Viral hepatitis and liver disease. New York: Alan R Liss: 497-500.**

6. **JILBERT AR, FREIMAN JS, BURRELL CJ, HOLMES M, GOWANS EJ, ROWLAND R, HALL P, COSSART YE (1988).** Virus-liver cell interactions in duck hepatitis B virus infection: a study of virus dissemination within the liver. **Gastroenterology 95: 1375-1382.**