HCV Specific Cell Mediated Immune Response in Health Care Workers

Thesis

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List of Abbreviations

Ag	Antigen
ALT	Alanine aminotransferase
APC	Antigen presenting cells
ARF	Alternative reading frame
AST	Aspartate aminotransferase activity
BrdU	Bromdeoxyuridine
CD	Cluster of differentiation
CDC	Centers for disease control and prevention
CFDA-SE	Carboxyfluorescein diacetate succinimidyl
CFSE	ester
CLDN-1	Claudin-1
CTL	Cytotoxic T lymphocyte
DC	Dendritic cells
DC-SIGN	Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
DMSO	Dimethyl sulfoxide
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme linked immunospot
ER	Endoplasmic reticulum
F	Frameshift
FACS	Fluorescence activated cell sorting
gC1qR	Globular domain of C1q receptor
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCWs	Health care workers
HIV	Human Immune Deficiency Virus
HS	Highly Significant
HVR	Hypervariable regions
HVR	Hypervariable region
ICS	Intracellular cytokine staining
IFN	Interferon

List of Abbreviations (Cont.)

IRES	Internal ribosome entry site
IRF	Interferon regulatory factor
LDLR	Low density lipoprotein receptor
L-SIGN	Liver/ Lymph node-specific intercellular adhesion molecule-3-grabbing integrin
MDC	Myeloid dendritic cells
MFI	Mean fluorescence intensity
MICA/B	MHC class-I related chain A/B
Mo-DC	Monocyte-derived dendritic cells
NCR	Non-coding region
NK	Natural killer cells
NKT	Natural killer T cells
NS	Nonstructural protein
NS	Non-Significant
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDC	Plasmacytoid dendritic cells
PHA	Phytohaemagglutinin
PI	Proliferation index
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
S	Significant
SPSS	Statistical Package for Social Sciences
SR-BI	Scavenger receptor class B type I
TCR	T-cell receptor
TGF	Transforming growth factor
TLR	Toll-like receptors
TNF	Tumour necrosis factor
Treg	Regulatory T cells
UTR	Untranslated region
WHO	World Health Organization

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Introduction

Hepatitis C virus (HCV) infection is an increasingly major public health problem, threat and concern worldwide ***(Yeung et al., 2001)***. There are 170 million infected individuals worldwide; the prevalence of infection is nearly 3% ***(Schaefer et al., 2004).***

Egypt has one of the highest prevalence rates of HCV infection in the world (16 – 18%) ***(Darwish et al., 2001).*** An important cause for the high exposure to HCV was the establishment of a large reservoir of infection as a result of extensive schistosomiasis control programs that used intravenously administered tartar emetic 20–50 years ago ***(Frank et al., 2000).***

Needlestick injuries of health care workers (HCWs) are an important occupational hazard leading to infections with blood borne pathogens like HBV, HCV, or HIV ***(Smith et al.,2006).*** Globally, more than 35 million HCWs face the risk of sustaining a percutaneous injury with a contaminated sharp object every year ***(Deisenhammer et al., 2006).*** Overall, the number of HCWs annually exposed to sharps injuries contaminated with HCV is estimated at 926,000 ***(Prüss-Üstün et al.,2005).***The transmission rate of HCV is estimated between

3 and 10% ***(Trim and Elliot, 2003)***. The risk of HCV-transmission increases by more than tenfold, with high levels of virus load of the source patient ***(Yazdanpanah et al.,2005).***

HCV infection is characterized by the high likelihood of chronicity after acute infection and significant risk of disease progression to cirrhosis and liver failure. Immune responses appear to be crucial in the control of infection, and patients with self-limited courses of acute HCV demonstrate coordinated activation of viral-specific CD4+ and CD8+T cells ***(Rehermann and Chisari, 2000).***

Few HCV infections resolve spontaneously but in this case, appear to afford protective immunity. Second HCV infections are usually shorter in duration and are less likely to persist, but mechanisms of virus control in immune individuals have not been identified. Clearance of the first infection takes 3–4 months and coincides with the delayed onset of CD4+ and CD8+ T cell responses ***(Shoukry et al., 2003).***

Aim of the Work

Detection of HCV specific cell mediated immune response in HCWs with no evidence of infection. (seronegative, negative Polymerase Chain Reaction (PCR), normal liver enzymes).

Chapter I

Hepatitis C Virus

Hepatitis C Virus

Introduction:

It has been 35 years since the first description of a chronic liver disease in blood transfusion recipients that was not caused by known viral hepatidites. Transfer of hepatitis to chimpanzees by blood products implicated in the human infections provided evidence for a new infectious agent of non-A, non-B hepatitis; physicochemical studies indicated it was a small, enveloped RNA virus (***Walker , 1999***).

The true scope of the public health problem was not recognized until 15 years later, when in 1989 the RNA genome of the hepatitis C virus (HCV) was molecularly cloned (***Choo et al., 1989***) and assays for serum antibodies to viral proteins were established (***Kuo et al., 1989***).

HCV infection was confirmed to be the leading cause of advanced liver disease and a major international public health problem. There are about 170 million chronic HCV carriers throughout the world. The global prevalence of chronic HCV infection averages 3 percent, ranging from 0.1 percent to 5 percent in different countries ***(Wang et al., 2003).***

I. Structure of Hepatitis C Virus:

HCV is the prototype virus within a new Hepacivirus genus of the family Flaviviridae ***(Choo et al., 1989).*** Other members of the family include Yellow fever virus,West Nile virus and Dengue Fever virus ***(Dufour, 2005).***

HCV has a positive strand RNA genome that is composed of a 5′-non-coding region (NCR), which includes an internal ribosome entry site (IRES), an open reading frame that encodes structural and non-structural proteins, and a 3′-NCR. The structural proteins, which form the viral particle, include the core protein and the envelope glycoproteins E1 and E2. The non-structural (NS) proteins include the p7 ion channel, the NS2-3 protease, the NS3 serine protease and RNA helicase, the NS4A polypeptide, the NS4B and NS5A proteins and the NS5B RNA-dependent RNA polymerase (RdRp) ***(Penin et al. ,2004).***

HCV has 6 major genotypes and more than 50 subtypes based on nucleotide diversity within the core, E1, and NS5 genes. HCV genotype may differ genetically from another by as much as 35 percent ***(Palmer, 2004).*** Three types of regions have been identified: highly conserved regions (e.g. the 5' untranslated region (UTR)), variable regions (e.g. E1 and NS5B) and hypervariable regions (HVR) (e.g. HVR1 and HVR2 in E2) ***(Genovese et al., 2005).***