Characterization of Differential Antibody Production against Hepatitis C Virus in Egyptian Chronic HCV Infection and Healthcare Workers

Thesis

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By

Doaa Essam Eldein Mahmoud Shalaby
MB, BCh
Ain Shams University

Supervised by

Prof. Dr. Mona Mohammed Rafik
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Dr. Dina Ahmed Soliman
Assistant Professor of Chemical & Clinical Pathology
Faculty of Medicine-Ain Shams University

Dr. Nesreen Ali Mohammed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
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Doaa Essam Eldein Mahmoud Shalaby

This work is dedicated to . . .

My beloved father, ***to whom I owe everything I ever did in my life and will achieve***

My mother ***for always being there for me***

My Aunt (Dr. Salwa Bakr) ***for her love and help.***

My brother ***and*** ***my sister*** ***for their support***

Finally ***my Husband and my lovely son*** ***for being the light of my life***

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List of Abbreviations

Abb.	Full term

Ab.....: Antibodies
APCs .....: Antigen-presenting cells
ARFP .....: Alternative open reading frame protein
CLDN-1.....: Claudin-1
CTL.....: Cytotoxic T lymphocyte
DC.....: Dendritic cells
E1 and E2.....: Envelop proteins
EIA.....: Enzyme immunoassay
ER .....: Endoplasmic reticulum
ERK .....: Extracellular regulated kinase
GAGs.....: Glucosaminoglycans
HCC .....: Hepatocellular carcinoma
HCV .....: Hepatitis C virus
HDL .....: High density lipoprotein
HIV .....: Human immunodeficiency virus
HVR .....: Hypervariable region
iDCs .....: Immature dendritic cells
IDU .....: Intravenous drug use
INF.....: Interferon
IPS-1 .....: IFN-β promoter stimulator protein 1
IRAK.....: Interleukin -1 receptor-associated kinase
IRF-3.....: IFN regulatory factor 3
ISDR .....: Interferon-α sensitivity-determining region
ISGs .....: IFN stimulated genes
ISREs.....: IFN-stimulated response elements
JNK.....: c-jun N-terminal kinases
MAPK .....: Mitogen activated protein kinases
mDC.....: Mature DCs

List of Abbreviations (Cont...)

Abb.	Full term
MHC	Major histocompatibility complex
MICA/B	MHC class-I related chain A/B
NAT	Nuclear acid testing
NK	Natural killer
NKT	Natural killer T cell
NS	Nonstructural proteins
OCLN	Occludin
OS	Oxidative stress
PAMPs	Pathogen recognition receptors
PD-1	Programmed death-1 receptor
PDCs	Plasmacytoid dendritic cells
PIAS	Protein inhibitor of activated STAT1
PKA	Protein kinase A
PP2A	Protein phosphatase 2A
RIBA	Recombinant immunoblot antibody assay
RIG-I	Retinoic acid inducible gene-I
ROS	Reactive oxygen species
RT-PCR	Reverse-transcription PCR
SPR	Surface plasmon resonance
SRB-1	Scavenger receptor type B class 1 protein
SVR	Sustained virological response
TCRs	T-cells receptors
TGF-β	Transforming growth factor β
TH1	T helper 1
TH2	T helper 2
TLRs	Toll like receptors
TMDs	Transmembrane domains
TNF-α	Tuomur necrotic factor α
Tregs	T regulatory cells

INTRODUCTION

Hepatitis C virus (HCV) infects >2% of the world population, with an estimated >500,000 new infections annually in the highest endemic country, Egypt ***(Miller and Abu-Raddad, 2010).*** In the United States, the rate of symptomatic HCV infection declined over the last decade and began to level out at ~ 4 million cases around 2005 ***(Wasley et al., 2007).*** Alarmingly, however, in developed countries, new cases are often associated with the younger age group (15-24 y) because of illegal injection drug use ***(Onofrey et al., 2011)***. Although some HCV-infected individuals can resolve infection without drug treatment, ~70% develop chronic hepatitis and, over a period of 20–30 y, 20–30% will develop liver cirrhosis and 1–5% hepatocellular carcinoma ***(Lemon et al., 2007).***

Hepatitis C virus (HCV) is classified in the *Hepacivirus* genus within the *Flaviviridae* family. The viral genome constitutes a 9.6-kb single-stranded positive-sense RNA with 5' and 3' non coding regions and a long open reading frame encoding a polyprotein precursor of about 3,000 amino acids in length. The HCV polyprotein precursor is co- and post-translationally processed by cellular and viral proteases to yield 11 viral proteins ***(Lindenbach and Rice, 2005)***. The structural HCV proteins include the core protein and transmembrane glycoproteins, E1 and E2. The core region also encodes for an alternative open reading frame protein (ARFP) or F protein

whose function is presently not known ***(Xu etal., 2001***). The region between the structural and nonstructural genes encodes for an integral membrane cation channel protein p7 ***(Griffin et al., 2003)*** which is essential for virus production ***(Jones et al., 2007).*** HCV has six nonstructural proteins; NS2, NS3 NS4A, NS4B, NS5A and NS5B ***(Moradpour et al., 2007).***

The humoral response to HCV infection is broadly targeted, with antibodies to both structural and non-structural proteins found in most cases. Although the commercial methodology to detect HCV-specific RNA and antibody responses in patient sera has greatly advanced in recent years there is no detailed information of the immunogenicity of different HCV proteins in patients suffering from chronic HCV infection ***(Sillanpää et al., 2009).***

On the other hand, healthy carriers of hepatitis C virus (HCV) infection exhibit a specific antibody response against HCV antigens, which could play a role in disease control. Detection of these antibodies may permit a thorough characterization of this response and further identify particular antibodies with potential clinical value ***(Barban et al., 2000).***

Aim of the Work

To determine qualitative differences in host antibody responses to different HCV proteins in Egyptian chronic HCV infection and healthy care workers and their correlation to clinical outcome.

Structure of Hepatitis C Virus:

HCV is a small (50 nm in size), enveloped, single-stranded, positive sense RNA virus. It is the only known member of the hepacivirus genus in the family Flaviviridae ***(Op De beek and Dubuisson, 2003) (Figure 1).***

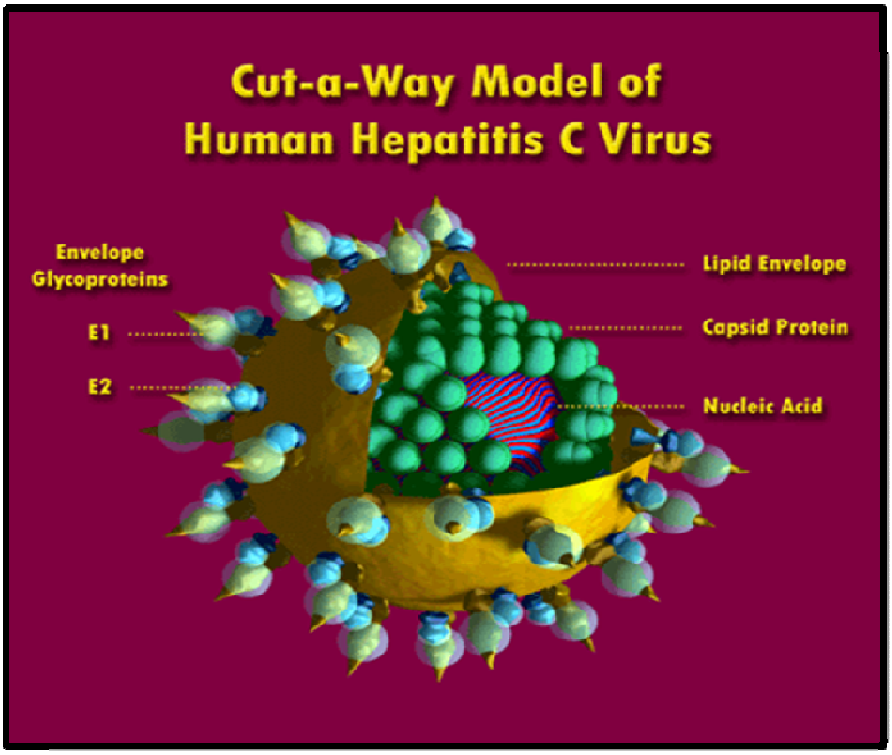

Figure (1): Structure of Hepatitis C Virus ***(www.hepcprimer.com).***

The viral genome constitutes a 9.6-kb single-stranded positive-sense RNA with 5' and 3' non coding regions and a long open reading frame encoding a polyprotein precursor of about 3, 000 amino acids in length. The HCV polyprotein precursor is co- and post- translationally processed by cellular

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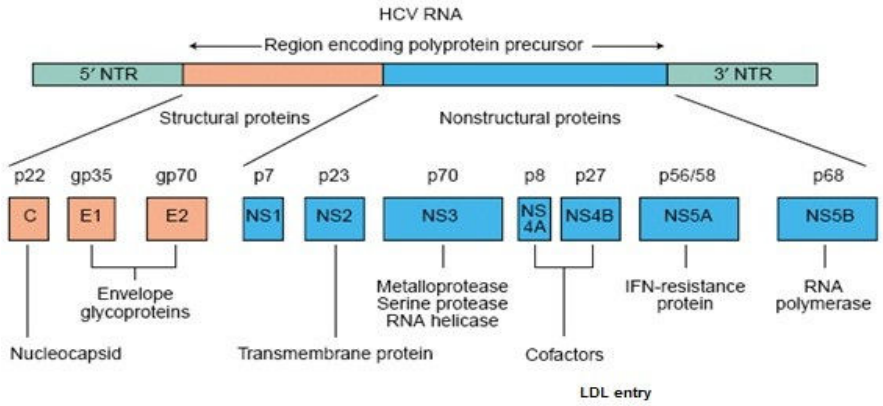

Figure (2): Proteins encoded by the HCV genome. HCV is formed by an enveloped particle harbouring a plus-strand RNA of 9.6 kb. The genome carries a long openreading frame (ORF) encoding a polyprotein precursor of 3010 amino acids. Translation of the HCV ORF is directed via a 340 nucleotide long 5' nontranslated region (NTR) functioning as an internal ribosome entry site; it permits the direct binding of ribosomes in close proximity to the start codon of the ORF. The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteases into ten different products, with the structural proteins (core (C), E1 and E2) located in the N-terminal third and the nonstructural (NS2-5) replicative proteins in the remainder. Putative functions of the cleavage products are shown ***(Beaulieu and Tsantrizos, 2004).***

HCV Structural Proteins

Core Protein

HCV core is a highly conserved basic protein which makes up the viral nucleocapsid. Core consists of HCV first 191 amino acids and can be divided into three domains on the basis of hydrophobicity: domain 1 (amino acids 1 - 117) contains mainly basic residues with two short hydrophobic regions, domain 2 (amino acids 118 - 174) is less basic and more hydrophobic and its C -terminus is at the end of p21, domain 3 (amino acids 175 - 191) is highly hydrophobic and acts as a signal sequence for E1 envelope protein ***(Bukh et al., 1994)***. Core protein can bind viral RNA ***(Santolini et al., 1994)*** via domain 1 (amino acids 1 - 74). Core is a cytosolic membrane-bound protein, which has been found to associate with the endoplasmic reticulum (ER), lipid droplets, mitochondria and the nucleus. It is also involved directly or indirectly involved in hepatocarcinogenesis and steatosis hepatitis ***(Hope et al., 2002).*** HCV core protein interacts with numerous cellular proteins and to affect host cell functions such as gene transcription, lipid metabolism, apoptosis and various signaling pathways ***(Tellinghuisen and Rice, 2002)***

Envelope Glycoproteins

HCV consist of two "envelop proteins" E1 and E2. These proteins are highly glycosylated and play an important role in cell entry. E1 serves as the fusogenic subunit and that E2 acts as the receptor binding subunit of the HCV envelope. The E1