A Cytokine Profile in Response to Stimulation of Peripheral Blood Mononuclear Cells by HCV C33

Thesis

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Summary

Hepatitis C virus (HCV) infection has become a global health problem with around 130–170 million infected people worldwide.

The majority of those infected enter a chronic disease state and further develop liver cirrhosis, hepatocellular carcinoma. Although combination therapy with pegylated interferon and ribavirin is available, only 55% of the infected patients show a favorable response.

This response greatly varies with the HCV genotype involved in the infection. Furthermore, the combination therapy is expensive and the associated side effects limit their usage.

HCV is a small, enveloped, positive sense single strand ribonucleic acid (RNA) virus of the family Flaviviridae consisting of the structural proteins (core, envelope 1 and 2) and the nonstructural proteins (NS2, NS3, NS4, NS5).

Most HCV proteins have a clearly defined role in the viral replication cycle and may also facilitate immune evasion by altering cell signaling pathways involved in host defense.

The NS3 is a multifunctional protein, with a serine protease which responsible for the polyprotein cleavage and RNA helicase/NTPase which is indispensable for RNA replication.

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List of Abbreviations

5-PL : Five-Parameter Logistic curve
aa : Amino acids
Ag : Antigen
ALT : Alanine aminotransferase
APC : Antigen presenting cells
ARF : Alternative reading frame
bDNA : branched DNA
CARs : Coxsackie virus B adenovirus receptors
CD : Cluster of differentiation
CDC : Centers for disease control and prevention
CFSE : Carboxyfluorescein diacetate succinimidyl ester
CLDN-1 : Claudin-1
CpG : Unmethylated cytosine preceding guanosine motif
CTL : Cytotoxic T lymphocyte
DC : Dendritic cells
DC-SIGN : Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin
EC1 : extracellular loop-1
EIA : Enzyme immuno assay
EIF2A : Eukaryotic translation initiation factor 2, alpha subunit
ELISA : Enzyme-linked immunosorbent assay
ELISpot : Enzyme linked immunospot
ER : Endoplasmic reticulum
F : Frameshift
FasL : Fasligand
GAG : Glycosaminoglycans
HBV : Hepatitis B virus
HCC : Hepatocellular carcinoma
HCV : Hepatitis C virus
HCWs : Health care workers
HDL : High density lipoproteins
HIV : Human Immune Deficiency Virus
HMW : High-molecular-weight
HS : Highly Significant

HVR : Hypervariable regions
IDU : Intravenous drug use
IFNγ : Interferon -gamma
IL2 : Interlukin2
IRES : Internal ribosome entry site
IRF : Interferon regulatory factor
ISDR : Interferon sensitivity determining region
ISDR : Interferon sensitivity determining region
JAMs : Junction–associated molecules
LD : Lipid droplets
LDL : Low density lipoprotein
LDLR : Low density lipoprotein receptor
LEL : Large extracellular loop
LPS : Lipopolysaccharide
L-SIGN : Liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin
M/M : Monocytes/macrophages
MAF : Macrophage activating factors
MDC : Myeloid dendritic cells
MHC : Major histocompatibility complex
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
NTPase : Nucleoside triphosphatase
NTR : Nontranslated regions
NTR IRES : Non translated region-internal ribosome entry site
ODN : Oligodeoxynucleotide
ORF : Open –reading frame
PAT : Parenteral antischistosomal therapy
PBMCs : Peripheral blood mononuclear cells
PCR : Polymerase chain reaction
PDC : Plasmacytoid dendritic cells
PE : Phycoerythrin
PEG-IFN α : Pegylated interferon-α

PI : Proliferation index
PKR : Protein kinase receptor
PM : Plasma membrane
RdRp : RNA-dependent RNA polymerase
RF : Replicative form
RFU : Relative fluorescence units
RI RNA : Replicative intermediate Ribonucleic acid
RIBA : Recombinant immuno blot assay
RIG-I : Retinoic-acid-inducible gene
RT-PCR : Real Time Polymerase chain reaction
Sig : Significant
SL : Stem loops
SPSS : Statistical Package for Social Sciences
SR-BI : Scavenger receptor class B type I
ssRNA : Single stranded genomic HCV RNA
STAT : Signal transducer and activator of transcription
SVR : Sustained virological response
TCR : T-cell receptor
Th : T helper cell
TLR : Toll-like receptors
TMA : Transcription mediated amplification
TNF : Tumour necrosis factor
TRAIL : TNF-related apoptosis-inducing ligand
Treg : Regulatory T cells
TRIF : Toll/interleukin-1 receptor domain-containing adapter inducing interferon-β
UTR : Untranslated region
VLDL : Very-low-density lipoproteins.
VL : Viral loads
WHO : World health organization

Introduction

Hepatitis C virus (HCV) infection is a major world health problem; In most cases (60–85 %), HCV infection progresses to chronic liver disease, which can lead to liver cirrhosis and hepatocellular carcinoma ***(Burlone and Budkowska, 2009).***

HCV is a small, enveloped virus. HCV has a single-stranded positive sense RNA genome, encoding a polyprotein of about 3000 amino acids, which is cleaved into structural (core, E1 and E2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins by host and viral proteases ***(Burlone and Budkowska, 2009).***

Hepatitis C viruses interfere with the complex cytokine network brought about by the immune system and liver cells in order to prevent an effective immune response, capable of viral control ***(Larrubia et al., 2009).***

Successful viral clearance is associated with a vigorous antiviral T-cell response. Both CD4 and CD8 T cells contribute to virus control ***(Chang et al., 2001)***.

CD4+ cell responses are critical to both the generation and maintenance of antiviral immune responses; they secrete cytokines that augment antibody production by B cells and prime CD8+ cells ***(Koziel, 2005).***

CD8+ T cells The effector function of CTLs consists of both the cytolysis of infected cells and the production of cytokines that lead to clearance of virus (e.g., TNF-α and IFN γ) ***(Koziel,2005).***

The imbalance between T helper cell Th1 (IL-2, IL-12, IFN γ) cytokines and Th2 (IL-10) cytokines affect the outcome of HCV infection. The defect in both IL-12and IFN γ production may contribute to persistence of HCV infection ***(Sarih et al., 2000)***.

Interferon γ (IFN- γ), a cytokine that plays an important role in inducing and modulating an array of immune responses, including the activation and differentiation of T cells, B cells, NK cells, macrophages. The secretion of IFN- γ is in response to IL-2 and IL-12 ***(Gattoni et al., 2006).***

Tumor necrosis factor (TNF)-α and IFN- γ and can purge viruses from infected cells noncytopathically. Also control viral infections indirectly, by modulating induction, amplification, recruitment, and effector functions of the immune response and by upregulating antigen processing ***(Guidotti and Chisari, 2001).***

Strong and persistent cell-mediated immune responses have been reported in HCV- seronegative individuals with documented exposure to HCV in the absence of detectable viral RNA ***(Post et al., 2004)***.

HCV-specific CD4+T cell responses persist in acutely infected individuals who permanently cleared the virus, and disappear in patients whose viraemia subsequently recurred ***(Chang et al., 2001)***.

HCV-specific CD8+T cell responses that are associated with spontaneous viral clearance tend to be multi-specific and polyclonal ***(Lauer et al., 2005)***.

Aim of the Work

The aim of the work is to detect a panel of cytokines IL2, IFN-γ and TNFα of cell culture supernatant from unstimulated and stimulated peripheral mononuclear cells (PBMCs) by HCV specific C33 peptide.

Hepatitis C Virus

I. Structure of Hepatitis C Virus:

The hepatitis C virus (HCV), identified in 1988 through molecular biological techniques, is an enveloped virus that is classified as a separate genus in the Flaviviridae family. The HCV genome is a single-stranded, positive-sense RNA molecule approximately 9.5 kilobases (kb) in length. Within an infected person, HCV exists as a population of closely related heterogeneous variants, called quasispecies, that result from the rapid development of mutations in the viral genome ***(Moyer et al., 1999).***

The putative infectious virus particle is composed of a nucleocapsid or ribonucleoprotein complex bearing the HCV genome; this inner structure is surrounded by a phospholipid bilayer, into which E1 and E2 envelope glycoproteins are anchored. However, infectious HCV particles become tightly associated with very-low-density lipoproteins (VLDL) during virus assembly and are (co-)secreted with VLDL ***(Burlone and Budkowska, 2009).***

Various forms of HCV virions appear to exist in the blood of infected individuals: virions bound to very low density lipoproteins (VLDL), low-density lipoproteins (LDL), immunoglobulins, and free circulating virions. Moreover, it is speculated that the association with LDL and/or VLDL protects the virus against neutralization by HCV-specific antibodies ***(Nahmias et al., 2006)***.