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A Cytokine Profile in Response to Stimulation of Peripheral Blood Mononuclear Cells by HCV C100 Peptide in Health Care Workers

Thesis

Submitted for Partial Fulfillment of Master Degree
in Clinical and Chemical Pathology

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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانكَ لاَ علِمَ لَنَا إِلاّ مَا

عَلّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

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Contents

List of abbreviations

ALT	Alanine aminotransferase
ARF	Alternate reading frame
CFSE	Carboxyfluorescein diacetate succinimidyl ester
CLDN-1	Claudin-1
CMI	Cell mediated immunity
Cryo EM	Cryo electron microscope
CTL	Cytotoxic T lymphocyte
Ds RNA	Double strand ribonucleic acid
DC	Dendritic cell
eIF	Eukarutic initiation factors
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
EIA	Enzyme immuno assay
GTPase	Glucose triphosphatase
HCC	Hepato-cellular carcinoma
HCV	Hepatitis C virus
HCWs	Health care workers
HDL	High density lipoprotien
HIV	Human Immune deficiency virus
HLA	Human leukocyte antigen
HVR	Hypervariable region
IRES	Internal ribosome entry site
JAK	Janus kinase
LDL	Low density lipoprotien
MDC	Myloid dendritic cells
MHC	Major histocompatibility complex
mRNA	Messenger Ribonucleic acid
NK	Natural killer cells
NKT	Natural killer T cells
NTR	Non translated region

OD	Optical density
ORF	Open reading frame
PAMP	Pathogen associated molecular pattern
PCR	Reverse transcription polymerase chain reaction
PD	Programmed death
PDC	Plasmacytoid dendritic cells
PI	Proliferation index
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
SCID	Severe combined immunodeficiency
SCID	Severe combined immunodeficiency
TLR	Toll-like receptors
TMA	Transcription mediated amplification
TMD	Trans-membrane domain
Treg	Regulatory T cells
UTR	Untranslated region
VLDL	Very-low-density lipoproteins.
WHO	World Health Organization

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Introduction

Hepatitis C virus (HCV) is one of the major causes of chronic hepatitis worldwide with an estimated 170 million being chronically infected ***(Lauer and Walker, 2001)***.

HCV infection is most often diagnosed by detecting virus-specific antibodies (anti-HCV); 75–80% of those having anti-HCV also have active infections with viremia, marked by the presence of HCV-RNA. Approximately 80% of infected individuals develop chronic hepatitis, among whom 20–30% may progress to hepatic cirrhosis and 2–3% of these go on each year to have hepatic failure and/or hepatocellular carcinoma ***(Seeff, 2002).***

HCV has been classified as the genus Hepacivirus in the Flaviviridae family. The HCV has 6 major genotypes and more than 50 subtypes ***(Simmonds et al., 2005).*** HCV is a positive sense single-stranded RNA virus with a genome of 9600 nucleotides encoding a single open reading frame (ORF) encoding a polyprotein of approximately 3000 amino acids that is processed during and after translation into at least 10 proteins ***(Lindenbach and Rice, 2005).*** HCV has structural proteins like core protein and envelope glycoprotein E1 and E2 as well as the non-structural (NS) proteins, which have essential functions in viral replication ***(Loyd, et al., 2007).*** The non structural proteins (NS) are NS2 (cysteine protease), NS3 (serine protease and helicase), NS4 (cofactor for serine protease), NS5a

(phosphoprotein) and NS5b (RNA-dependent RNA polymerase) ***(Lindenbach and Rice, 2005)***.

CD4+ T-cell proliferates and cytokines are secreted in response to a panel of recombinant HCV antigens including C100. All patients with self limited disease had a significant CD4+ T-cell proliferation to C100, running parallel with the antigen-stimulated secretion of IL-2 and IFN-γ but not with IL-4 and IL-10, indicating predominant Th1 response **(*Semmo and Klenerman, 2007).***

An acute viral infection triggers the activation of several antiviral effectors. This innate antiviral response is an early host defense mechanism that occurs prior to adaptive immune responses ***(Katze, 2002).***

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).***

The role of $CD4^+$ T cells in acute HCV infection has been examined, in which the loss of $CD4^+$ T cells resulted in persistent infection ***(Grakoui et al., 2003).*** Moreover, $CD4^+$ T cell levels also appear to be important during acute HCV infection, as the level of $CD4^+$ T cell proliferative responses is associated with viral clearance ***(Aberle et al., 2006).***

CD8 T cells could respond to HCV viral infection through 2 main mechanisms: the killing of infected hepatocytes or the secretion of antiviral cytokines ***(Lauer et al., 2005).***

Cytokines are important for the clearance or persistence of viremia. Cytokines are produced by a vigorous viral-specific helper T cells response ***(Aberle et al., 2006).***

CD4-derived IL-2 may be one of the factors required during the primary immunization with HCV to program the differentiation of fully functional cytotoxic T lymphocytes memory ***(Williams and Bevan, 2007)***.

TNF-α is the principal mediator of the acute inflammatory response to infectious pathogens and is responsible for many of the systemic complications of severe infections ***(Abul Abbas and Lichtman, 2005).***

Gamma interferon (IFN-γ) is closely associated with control of many viruses and other intracellular pathogens (***Novelli and Casanova, 2004).***

Aim of the Work

The aim of the work is to detect the level of a panel of cytokines IL2, IFN-γ and TNF-α in cell culture supernatant from unstimulated and stimulated peripheral blood mononuclear cells (PBMCs) by HCV specific C100 peptide of health care workers (HCWs).