

لمحة عن استجابة محركات الخلايا بعد تحفيز الخلايا الطرفية وحيدة النواة باستخدام الببتيد سى 100 للفيروس الكبدى سى للعاملين بالخدمات الصحية

رسالة

توطئة للحصول على درجة الماجستير
في الباثولوجيا الاكلينيكية والكيميائية

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2011

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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2011

بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا

عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

سورة البقرة آية (32)



Acknowledgement

All praise be to Allah and all thanks. He has guided and enabled me by His mercy to fulfill this thesis, which I hope to be beneficial for people.

*I would like to express my deepest gratitude and sincere appreciation to **Prof. Dr. Mona Mohamed Rafik**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her continuous encouragement, her kind support and appreciated suggestions that guided me to accomplish this work.*

*I am also grateful to **Dr. Khaled Omar Abdallah**, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University who freely gave his time, effort and experience along with continuous guidance through out this work.*

*Special thanks are extended to **Dr. Dina Ahmed Soliman**, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University for her constant encouragement and advice whenever needed.*

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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 (*Lloyd, 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).