Assessment of conserved peptides within the envelope (E2) glycoprotein of hepatitis C virus as candidates for development of neutralizing antibodies

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بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم

صدق الله العظيم

Dedication

I dedicate this work

To my family:

My Kind Mum

My Great Dad

My Beloved Sister

My Dearest Husband

My sweethearts Hamdy & Farida

I thank God for choosing you to be my family

Thank you for supporting me

With kindness, patience, patience, patience and love

Yours,

Yasmine Sayed El Abd

To My Dear Husband

I am very grateful & pleased with
Everything you do for me

Thanks a lot for your
Encouragement
Thoughtfulness
Assistance
Patience
Care
And
Love

To My little angles

Hamdy & Farida

I hope one day,
You read this thesis
& be proud that
I'm your mother

Thanks God
For the Gorgeous
& Precious Gift
My sweethearts

Hamdy & Farida

Who fill my life with
Happiness
Smiles &
Blesses

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Abstract

The reason(s) why human antibodies raised against hepatitis C virus do not offer protection against multiple viral infections may be related to either genetic variations among viral strains, low titers of anti E2 antibodies or interference of none or low neutralizing antibodies with the function of neutralizing antibodies. This study was designed to assess the immunogenic properties of genetically conserved peptides derived from envelope E2 region and test their neutralizing activities to determine the possibility of their development to potential therapeutic and/or prophylactic vaccines against HCV. Goats immunized with E2 conserved synthetic peptides termed Pep.36 (a.a 430-447), Pep.37 (a.a 516-531) and Pep.38 (a.a 412-420) generated high titers of antibody responses higher than comparable titers of antibodies to the same epitopes in chronic HCV patients. Also Pep.38 elicited approximately 2 fold increase in cell proliferation of specific antibody-secreting peripheral blood mononuclear cells from immunized goats. When using *invitro* culture model of Huh7 cell lines for infection and neutralization experiments antiPep.37 and anti Pep.38 were proven to be neutralizing to HCV particles in sera from patients infected predominantly with genotype 4a (75% & 87.5% respectively). On the other hand antiPep.36 exhibited weak viral neutralization capacity on the same samples (31.25%). Taking together the results of humoral immunity and cellular responses suggest that E2 conserved peptides Pep.37 and Pep.38 represent essential components of a candidate peptide vaccine against HCV infection

Key words

Hepatitis C virus (HCV)**,** anti E2 antibodies**,** neutralizing antibodies**,** invitro culture model for HCV**,** candidate peptide vaccine for HCV.

List of Abbreviations

a.a	Amino acid
Abs	Antibodies
BCIP/NBT	5-Bromo-4-Chloro-3'-Indolyphosphate p-Toluidine Salt / Nitro-Blue Tetrazolium Chloride
bDNA	Branched DNA
bp	Base pair
BSA	Bovine Serum Albumin
C	Cytidine
CD	Cluster of differentiation
Co_2	Carbon dioxide
DAB	Diaminobenzidine
ddH_2O	Double distilled water
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
DNA	DeoxyripoNucleic Acid
dNTP	Deoxynucleoside Triphosphate
Dot-EIA	Dot Enzyme Immunoassay
E	Envelop
E1	Envelope protein 1
E2	Envelope protein 2
ECMV	Encephalomyocarditis virus
ELISA	Enzyme Linked Immunosorbent Assay
EMCV	Encephalomyocarditis
ER	Endoplasmic Reticulum
FACS	Fluoresence Activated Cell Sorting
FBS	Fetal Bovine Serum
FCS	Fetal Calf Serum
FITC	Fluorescein isothiocyanate
GAGs	Glycosaminoglycans
gps	glycoproteins
GTP	guanosine triphosphate
HAV	Hepatitis AVirus
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCVcc	Cellular clone of HCV
HCVpp	Hepatitis C Virus pseudoparticles
HDL	High Density Lipoprotein