Role of Natural killer T Cells in Obesity-Related Liver Disease

Thesis

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Heba Nabil Mohamed

Contents

List of Abbreviations

ACAID	anterior chamber-associated immune deviation
~-GalCer	alpha-galactosylceramide
ALD	alcoholic liver disease
ALT	Alanine aminotransferase
AP-1	activator protein-1
APCs	antigen-presenting cells
apo B	apolipoprotein B
APOC3	apolipoprotein C3
aPTT	activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
AUC	area under carve
BMI	body-mass index
CDD	choline-deficient ethionine-supplemented diet
CD	Cluster of differentiation
CRP	C reactive protein
CI	confidence interval
CT	computerized tomography
CTL	cytotoxic T lymphocyte
DCs	dendritic cells
EAE	experimental allergic encephalomyelitis
EDTA	Ethylene Diamine tetra-acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FBS	Fasting blood sugar
FAs	fatty acids
FFAs	Free fatty acids
FITC	fluorescine isothiocyanate
FP-1	ferroprotein-1
GATA3	guanosine adenosine thymidine adenosine 3
GLUT-4	Glucose transporter type-4
GSL	glycosphingolipid
HCC	hepatocellular carcinoma
HCV	Hepatitis C virus
HDL	High-density lipoproteins

HFE	haemochromatosis gene
HIV	human immune deficiency virus
^{1}H MRS	Proton Magnetic Resonance Spectroscopy
HOMA-IR	Homeostasis model assessment of insulin resistance
HRP	horse radish peroxidase
HS	highly-significant
hs-CRP	High sensitivity C-Reactive Protein
IFG	impaired fasting Glucose
IFN-¥	Interferon Gama
iGb3	Isoglobotrihexosylceramide
IL	Interleukin
IQR	interquartile range
iNKT	invariant Natural killer T
KCs	kuffer cells
LDL	low-density lipoproteins
LFC	liver fat content
LPS	lipopoly saccharide
LR	likelihood ratio
MoAb	monoclonal antibodies
MHC	Major histocompatibility
mRNA	messenger ribonucleic acid
MRS	Magnetic Resonance Spectroscopy
MTP	microsomal triglyceride transfer protein
NAIP	neuronal apoptosis inhibitory protein 2
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NF-*k*B	nuclear factor *k*B
NIDDM	Non-insulin dependent diabetes mellitus
NK	natural killer
NKT cell	Natural killer T cell
NS	non-significant
ORLD	obesity related liver disease
PBS	Phosphate buffered saline
P C	personal computer
PE	phycoerythrin
PPARs	peroxisome proliferator-activated receptors

PT	Prothrombin Time
RNS	reactive nitrogen species
ROC	receiver-operating characteristic
ROS	reactive oxygen species
S	significant
SPSS	Statistical Package for Special Sciences
SREBP	sterol regulatory element binding protein
t	Student's t-test
T-bet	T-box expressed in T cells
TCR	T cell receptor
T2DM	type 2 diabetes mellitus
TFS	transferrin saturation
TGF-β	Tumor Growth Factor Beta
Th	T helper
TLR	toll like receptors
TMB	tetramethylbenzidine
TNF-~	Tumor Necrosis Factor Alpha
TRAIL	TNF-related apoptosis-inducing ligand
UCP2	un-coupling protein C
VLDL	very low-density lipoproteins
χ^2	Chi-square test

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Introduction

Liver involvement in obesity falls within a clinical entity called nonalcoholic fatty liver disease (NAFLD), characterized by macrovesicular liver steatosis in absence of significant alcohol consumption. Potential steps involved in the pathogenesis of NAFLD include abnormalities of lipid metabolism, production of reactive oxygen species, increased hepatic lipid peroxidation, activated stellate cells, and abnormal patterns of cytokine production. According to the multihit theory, the first hit involves accumulation of fat in the hepatic parenchyma, probably due to insulin resistance commonly observed in patients with NAFLD. Later, leptin has been involved in the pathogenesis of NAFLD ***(Iorio et al., 2006).***

NAFLD affects 10–20% of the population in developed countries and is increasing in prevalence with the rise of diabetes and obesity. The molecular mechanisms underlying the pathogenesis of NAFLD remain largely unknown. However further studies suggest that natural killer T (NKT) cells may have a protective effect in animal models of NAFLD ***(Li. et al., 2005).***

High-fat diet consumption, and feeding on a sucrose diet cause NAFLD associated with reduction of hepatic NKT cells. This reduction may be a result of reduced hepatic cluster of differentiation 1d (CD1d) expression and increased NKT apoptosis caused by reduced production of nor epinephrine and interleukin-15 (IL-15) ***(Gao et al., 2009).***

Depletion of NKT cells promotes proinflammatory polarization of hepatic cytokine production that sensitizes the liver to lipopoly saccharide (LPS) toxicity, whileas elevation of hepatic NKT cells by probiotic treatment or adoptive transfer improved NAFLD ***(Ma et al., 2008).***

However, findings from clinical studies of NKT cells in patients with NAFLD have been controversial. It was reported that peripheral NKT cells are depleted in patients with NAFLD and correlated negatively with disease severity ***(Xu et al., 2007)***. On the other hand, ***Tajiri et al. (2009)*** reported that hepatic NKT cells are increased in NAFLD and may promote liver injury.

Aim of the Work

The aim of the present study is to estimate the percentages of NKT cells in the peripheral blood of patients with obesity related liver disease and evaluate its role in the disease pathogenesis.

Nonalcoholic Fatty Liver Disease

Introduction:

Liver involvement in obesity falls within a clinical entity called NAFLD, characterized by macrovesicular liver steatosis in absence of significant alcohol consumption. Potential steps involved in the pathogenesis of NAFLD include abnormalities of lipid metabolism, production of reactive oxygen species, increased hepatic lipid peroxidation, activated stellate cells, and abnormal patterns of cytokine production. According to the multihit theory, the first hit involves accumulation of fat in the hepatic parenchyma, probably due to insulin resistance commonly observed in patients with NAFLD. Later, leptin has been involved in the pathogenesis of NAFLD ***(Iorio et al., 2006).*** Low adiponectin levels and increased leptin, as usually observed in obese patients, may predispose to ectopic fat deposition in liver ***(Rossi et al., 2011).*** People who carry the variants of a gene for apolipoprotein C3 (APOC3), which produces an enzyme needed for proper fat metabolism, have a higher incidence of both NAFLD and insulin resistance ***(Elaine and Moore, 2012).***

The condition of liver lipid accumulation, resembling alcohol-induced injury but occurring in patients who do not use alcohol or maximum 2-3 glasses/day, is called NAFLD ***(Neuschwander-Tetri and Caldwell, 2003).*** Several often

incorrectly used synonyms for the same disease are diabetes hepatitis, fatty-liver hepatitis, alcohol-like liver disease, Laennec's disease and nonalcoholic steatohepatitis (NASH) ***(Angulo, 2002).*** The term nonalcoholic is used because NAFLD and NASH occur in individuals who do not consume excessive amounts of alcohol. Yet, in many respects, the histological picture of NAFLD is similar to what can be seen in liver disease that is due to excessive intake of alcohol. However, the clinical circumstances in NAFLD and NASH are very different from those in alcoholic liver disease (ALD) ***(Elaine and Moore, 2012).*** NAFLD disease is an increasingly recognized condition, fuelled by the increasing prevalence of obesity, and is very rapidly becoming a major health problem world-wide ***(Angulo, 2007 and Browning et al., 2004)***.

NAFLD in the early stage is usually asymptomatic ***(Adams and Lindor, 2007)***, but it is by itself a risk factor for hepatocellular carcinoma (HCC) and is also part of the natural (progressive) history of nonalcoholic steatohepatitis (NASH), which can lead to cryptogenic fibrosis ***(Bugianesi, 2007)***. Due to this risk of progression to more severe liver disease through the consequences of its fibro-inflammatory risk, NAFLD has been predicted to be the major cause of liver transplantation in 2020 ***(Charlton, 2004)*** stressing the great need for early detection of the disease.