Assessment Of Tissue $CD4^+$ Foxp3 And $CD8^+$ T-Cells In Non Segmental Vitiligo

Thesis

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تقييم الخلايا الليمفاوية ت $CD4^+$ $CD8^+$ FoxP3 فى الأنسجة فى حالات البهاق المنتشر

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LIST OF ABBREVIATIONS

6BH4	Tetrahydrobiopetrin
ACTH	Adrenocorticotrophic hormone
ADCC	Antibody dependent cellular cytotoxicity
AGRP	Agouti related peptide
AP	Alkaline phosphatase
APS1	Autoimmune polyendocrine syndrome type 1
ASIP	Agouti signaling peptide
BC	Before christ
Bcl-2	B cell leukemia/lymphoma 2 gene
BFGF	Basic fibroblast growth factor
B-FGFR	Basic fibroblast growth factor receptor
BSA	Body surface area
CCL	Chemoattractant ligand
CCR	Chemokine receptor
C-MET	Proto antigen encodes HGF
CTLA4	Cytotoxic T lymphocyte antigen 4
DC	Dendretic cells
DCT	Dopachrome tautomerase
DEJ	Dermo-epidermal junction
DI	Distilled water

DNA	Deoxyribonucleotide acid
DOPA	Dihydroxiphenolalanine
EDTA	Ethylenediaminetetraacetic acid
ET-1	Endothelin
ETBR	Ethidium bromide
FLIP	Flagellar biothynthesis protein gene
FOXP3	Forkhead box p3
GITR	Glucocorticoid induced tumar necrosis factor receptor
GM-CSF	Granulocyte macrophage colony-stimulating factor
H_2O_2	Hydrogen peroxide
HGF	Hepatocyte growth factor
HIER	Heat induced epitope recovery
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
HSP	Heat shocked proteins
ICAM	Intercellular adhesion molecules
IDDM	Insulin dependent diabetes mellitus
IFN-γ	Gamma interpheron
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL-6	Interleukin 6
IPEX	Immunodysregulation polyendocrinopathy

	enteropathy x-linked syndrome
KGF	Keratinocyte growth factor
KGFR	Keratinocyte growth factor receptor
L	Lesional
LRP	Leucine rich repeat
MART1	Melanoma antigen recognized by T cells 1
MC	Melanocortin
MCHR1	Melanin concentrating hormone receptor 1
MCI-R	Melanocortin Receptor
MHC	Major histocompatibility complex
mRNA	Messenger ribonulcleotide acid
MSH	Melanocyte stimulating hormone
NACHT	Neural apoptosis inhibitory protien
NADPH	Nicotinamide adenine dinucleotide phosphate
NALP1	NACT,LRP- and PYD containing proteins
NK	Natural killer cells
NL	Non lesional
NSV	Non- segmental vitiligo
PAH	Polycyclic aromatic hydrocarbon
PBMCS	Peripheral blood mononuclear cells
PBS	Protein blocking serum
PCR	Polymerase chain reaction

PL	Perilesional
Pmel17	Melanosomal matrix protein
POMC	Proopiomelanocortin
PYD	Pyurin N-terminal homology domain
ROS	Reactive oxygen species
SCF	Stem cell factor
SNPs	Single nucleotide polymorphism
SOX	Sex determining region y -box
TBST	Tris buffered saline +0.1 % tween 20
TGF-β	Transforming growth factor beta
Th	T helper cells
TNF-γ	Tumar necrosis factor gamma
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	T regulatory cells
TRP	Transient receptor potential channel
TYRP1	Tyrosinase related protein 1
UV	Ultra violet
UVB	Ultra violet band

INTRODUCTION

Vitiligo is a depigmenting disorder characterized by the loss of melanocytes from the epidermis. It strikes approximately 0.5-1% of the world population ***(Taieb et al., 2007).*** Although the exact etiology of vitiligo has not yet been established, an autoimmune component plays a major role in its pathogenesis. In progressive disease the $CD4^+/CD8^+$ ratio is decreased among skin-infiltrating T cells and $CD8^+$ T cells isolated from vitiligo skin are cytotoxic to melanocytes ***(Wankowiccz-Kalinska et al., 2003).***

Cytotoxic T cells require $CD4^+$T cell help to be activated against melanocytes ***(Steitz et al., 2005).*** Involved helper and cytotoxic T cells from progressing vitiligo margins generate predominantly type 1 cytokines, namely, interferon (IFN)-γ and tumor necrosis factor (TNF)-α suggesting that vitiligo is a Th1-mediated disease ***(Le Poole et al., 2004).*** Autoimmunity results from unchecked control by regulatory T-cells (Tregs)**,** although the exact reason for autoimmune diseases is not yet fully elucidated ***(Levings et al., 2001).***

Native $CD4^+$ helper T cells are known to develop into at least 4 types, namely-helper 1(Th1), Th2, Th17, and regulatory T cells (Tregs). Th1 cells primarily produce IFN-γ and TNF-α ;Th2 cells synthesize interleukin (IL)-4, IL-5, IL-13 ; Th17 cells produce IL-17 and IL-6; and Tregs synthesize IL10 and transforming growth factor (TGF)-β ***(Ortonne et al., 2003).***

Markers expressed by Tregs include FoxP3, GITR, CTLA-4 and CD25, yet only FoxP3 expression is relatively unique to regulatory T cells ***(De Boer et al., 2007).*** FoxP3 is the acronym given to the forkhead winged-helix transcription factor "forkhead box P3" which serves as the dedicated mediator of the genetic program governing Tregs development and function; therefore it is regarded as a reliable and specific marker for Tregs ***(Hori, 2003; Yagi et al., 2004).***

Tregs have several immunomodulatory effects such as an anti-inflammatory role to maintain tolerance to self–antigens. In addition, Tregs have the ability to inhibit the responses of $CD8^+$ T cells, natural killer cells and $CD4^+$ T cells, so that Tregs may be important in the prevention of autoimmune diseases ***(Afzali et al., 2007).***

The role of Tregs in the pathogenesis of vitiligo is still disputable because some authors say that the serum level of Tregs increase ***(Abdallah et al., 2009).*** Others revealed that The percentage of $CD8^+$ T cells was significantly increased in vitiligo group compared with the controls and no difference was detected between the patients and control groups in percentages of $CD4^+$ T cells ***(Basak et al., 2008)*** While others revealed no systemic drop in the abunance or activity of Tregs in vitiligo patients and also reported the percentage of Tregs among skin infiltrating T cells are drastically reduced in non-lesional, perilesional and lesional vitiligo skin ***(Klarquist et al.,2010).***