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

Thesis

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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

H₂O₂ 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 antiinflammatory 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).