Expression of Toll-Like Receptor 9 in Verruca Vulgaris

Thesis

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By

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Abstract

Recent studies indicate that several Toll-like receptors (TLRs) are implicated in recognizing viral structures and instigating immune responses against viral infections. Warts are benign proliferations of skin and mucosa caused by the human papillomavirus (HPV). The effect of cryotherapy on wart clearance may be through necrotic destruction of HPV-infected keratinocytes or by inducing local inflammation that triggers an effective cell-mediated response The aim of this study is to detect the expression of TLR 9 in verruca vulgaris & the possible changes that may occur in TLR 9 expression in adjacent warts after treating one of them. Polymerase chain reaction (PCR) and In-situ PCR of skin samples were performed to determine the expression and localization of mRNA of TLR 9. In normal human skin, the level of TLR9 was low. In veruuca vulgaris lesions, the level of TLR9 was very high. After cryotherapy it was but not reach to normal skin level. Likewise, In-situ PCR indicated that keratinocytes of epidermis in NS little expressed TLR9, whereas TLR9 was strongly expressed on the epidermis and dermis of VV lesions. In addition, the mRNAof TLR9 was moderate expression in the VV after cryotherapy. Our results indicated that TLR9 may have an important role in cutaneous innate immune responses to cutaneous human papillomavirus infections and the using of cryocautery in treatment of warts may enhance the immune response against the viral infection.

Keywords:

Toll-like receptors; TLR9; Verruca Vulgaris; Cryotherapy.

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LIST OF ABBREVIATIONS

ALA	Aminolevulenic acid
APCs	Antigen presenting cells
CD	Cluster of differentiation
CMV	Cytomegaloviru
CO_2	Carbon dioxide
CpG	Cytosinphosphatidyl- Guanosin
CpG-ODNs	CpG-containing oligodeoxynucleotides
DNA	Deoxyribonucleic acid
DNCB	Dinitrochlorobenzene
DPCP	Diphenylcyclopropenone
ds-DNA	Double stranded deoxyribonucleic acid
Er:YAG	Erbium Yttrium- Aluminum- Garnet
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen system
HMGB	High mobility group box 1 protein
HPV	Human papillomavirus
HSPs	Heat shock proteins
HSV	Herpes simplex virus
IFNs	Interferons
IFN-γ	Interferon gamma
IKK	Inhibitor kabba B (IκB) kinase
IL	Interleukin
IL-1Rs	Interleukin-1 receptors
IRAK	Interleukin-1 receptor associated kinase

IRF	Interferon regulatory factor
IκB	Inhibitor kabba B
LAM	lipoarabinomanna
LCs	Langerhans cells
LLRs	Leucine-rich repeats
LNCaP	Androgen-sensitive human prostate adenocarcinoma cells derived from the left supraclavicular lymph node metastasis from a 50-year-old caucasian male
LPS	Lipopolysaccharide
MBL	Mannose-binding lectin
MHC	Major histocompatibility complex
mRNA	Messenger RNA
MyD88	Myeloid differentiation factor 88
NF-κB	Nuclear factor- kappa B
NOD	Oligomerization domain
Opn	Osteopontin
PAMPs	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
pDCs	Plasmacytoid dendritic cells
PHA	Phyto-haemagglutinin
PPD	Purified protein derivative
PRRs	Pattern-recognition receptors
RNA	Ribonucleic acid
SADBE	Squaric acid dibutyl este
SARM	Sterile alpha and HEAT/Armadillo motif
SLE	Systemic Lupus erythematosus

ssRNA	Single strand Ribonucleic acid
TAK1	Transforming growth factor-β associated kinase 1
TBK	Tank binding kinase
TDLN	Tumor draining lymph nodes
TGF-α	Ttransforming growth factor alfa
TGF-β	Transforming growth factor beta
TIR	Toll/ interleukin-1 receptors (IL-1R)
TIRAP	TIR domain-containing adaptor protein
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor alfa
TRAF	Tumor necrosis factor receptor associated factor
TRAM	TRIF-related adaptor molecule
TRIF	TIR-containing adaptor inducing interferon regulatory factor b
VLP	Virus like particles

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Introduction

INTRODUCTION

Warts are benign proliferations of skin and mucosa caused by the human papillomavirus (HPV). Currently, more than one hundred types of HPVs have been identified. Certain HPV types tend to occur at particular anatomical sites; however, warts of any HPV type may occur at any site ***(Rivera and Tyring, 2004).***They may exist in different forms; common warts (verruca vulgaris), plane or flat warts (verruca plana), genital warts (condyloma acuminata), and plantar warts (verruca plantaris) ***(Lipke, 2006).***

Treating warts is a therapeutic challenge for most physicians. No single therapy has been effective in achieving complete remission in every patient. ***(Sterling, et al., 2001).***

Common and plantar warts may regress spontaneously from one to two years. Therapeutic options include cryotherapy with liquid nitrogen, electrosurgery, laser ablation, intralesional bleomycin, salicylic acid, cimitidine, interferone, imiquimod, or sensitization with a compound such as a candida antigen. Topical irritants like tretinoin may be effective in flat warts. Anogenital warts need to be followed carefully due to the risk of squamous cell carcinomatous changes ***(Kodner and Nasraty, 2004).***

Cryotherapy is available for the treatment of verruca vulgaris in primary care and dermatology offices. It is considered a second-line therapy ***(Leman and Benton, 2000).*** The effect on wart clearance may be through necrotic destruction of HPV-infected keratinocytes or by inducing local inflammation that triggers an effective cell-mediated response ***(Stulberg and Hutchinson, 2003).***

Toll-like receptors (TLRs) are mammalian homologues of Toll, which was originally identified in Drosophila ***(Rocket al., 1998).***

Toll-like receptors (TLRs) represent a family of type I transmembrane proteins that are characterized by an extracellular leucine-rich repeat domain and a cytoplasmic domain similar to interleukin (IL)-1 receptor **(*Belvin & Anderson 1996).*** TLRs mediate an innate immune response by directly recognizing pathogen-associated molecular patterns (PAMPs) that are common among a large group of pathogens like, bacterial cell-wall components (such as lipopolysaccharide (LPS), peptidoglycan (PGN), and lipoteichoic acid), fungal cell wall (zymosan), viral double-stranded RNA molecules, and unmethylated CpG DNA (***Aderem & Ulevitch 2000 ; Akira et al., 2001***). Toll-like receptors (TLRs) have been shown to be a major class of PRRs because they can respond to different PAMPs ***(Medzhitov and Janeway, 2000 & Medzhitov, 2001).***

In mammals, at least, 13 identified TLRs ***(Roach et al., 2005)*** with distinct specificities to recognize highly conserved structural motifs of microbial pathogens as well as several host-derived molecules been identified ***(Akira and Takeda, 2004).*** There are at least 10 TLRs in humans and 13 in mice. Both humans and mice have TLRs 1–9. TLR10 is only found in humans, whereas TLR11 is only found in mice ***(West et al., 2006).***

TLR9 can be activated by unmethylated CpG DNA derived from bacterial and viral genomes ***(Lund et al., 2003).***

Verruca vulgaris skin lesions expressed TLR3 and 9 in addition to IFN-β and TNF-α . These viral-induced proinflammatory cytokines may play a pivotal role in cutaneous innate immune responses. ***(ku et al., 2008).***

- **Aim of the work:**

The aim of the work is to detect the expression of TLR 9 in verruca vulgaris & the possible changes that may occur in TLR 9 expression in adjacent warts after treating one of them by cryotherapy.

Review of literature
Chapter 1: Warts

CHAPTER 1: WARTS

HISTORY

Cutaneous warts were known to the ancient Greeks and Romans in the first century. Genital warts were believed to be a form of syphilis or gonorrhea until the twentieth century. In 1907, the viral nature of warts was established by Ciuffo. The viral particle was observed in 1949, using an electron microscope. Eighty three types have been isolated in 1976, but then more than one hundred and thirty types of human papillomaviruses (HPVs) have been identified based on polymerase chain reaction (PCR) ***(Nebesio et al., 2001).***

Papillomaviruses were derived from the Latin word in which the Greek prefix "papilla" means nipple or pustule while the Greek suffix "oma" means tumor ***(Lyell, 1966).***

Human papillomaviruses, which represent the causative virus of warts, are members of the Papovaviridae family (***Zheng and Baker, 2006).***

PATHOGENESIS

- **VIROLOGY**

Human papillomavirus is a double stranded (ds) deoxyribonucleic acid (DNA) virus which is fifty five nanometer in diameter. Its capsid lacks an envelope, making HPVs very stable, infectious for years, and resistant to many therapeutic agents. The HPVs ds-DNA genome is composed of eight thousands nucleotide base pairs, which encode eight gene proteins: six are early and known as (E) genes and