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Samenvatting (Dutch Summary)

Herpetische stromale keratitis (HSK), met name veroorzaakt door herpes simplex virus type 1 (HSV-1), is de meest voorkomende oorzaak van non-traumatische cornea blindheid in de wereld. In tegenstelling tot herpetische epitheliale keratitis (HEK), waarbij de cornea epitheel lesies veroorzaakt worden door het cytopathologisch effect van het virus, wordt de pathologie van de chronische hoornvliesontsteking HSK veroorzaakt door infiltrerende onstekings-cellen in het stroma en de sub-epitheliale cellaag van de cornea. Herpetische keratitis (HK) is een complexe ziekte, waarbij zowel het virus als de gastheer bepalend zijn voor het verloop van de ziekte.

Het ontstekingshormoon Granulocyt Macrofaag Kolonie Stimulerende Factor (GM-CSF) speelt een belangrijke rol bij chronische ontstekingsprocessen zoals reuma en astma. In **hoofdstuk 2** is de rol van GM-CSF in de immunopathogenese van HSK onderzocht. De studie laat zien dat menselijke hoornvlies epitheel cellen en fibroblasten GM-CSF uitscheiden na stimulatie met zowel macrofaag- als T lymphocyt-specifieke ontstekings-hormonen (o.a. tumor necrosis factor alfa, interleukine 1 en 17). GM-CSF zorgt dat granulocyten langer blijven leven en dat ze geactiveerd worden, waardoor ze HSV-1-geïnfecteerde hoornvlies fibroblasten kunnen doden. Blokkering van GM-CSF in cornea's van HSK patiënten dient te worden overwogen om de prognose van HSK patiënten in de toekomst te verbeteren.

Voorgaande studies in muizen hebben aangetoond dat de mate van HK afhankelijk is van de virus stam waarmee de muis geïnfecteerd wordt. In **hoofdstuk 3** is een groot aantal hoornvlies HSV-1 kweken van HK patiënten onderzocht om te bepalen wat de prevalentie is van de drie bekende HSV-1 glycoprotein G (gG) en gI varianten en of een variant geassocieerd is met het HK ziektebeeld. De HSV-1 variant B kwam het meeste voor. Er werden geen klinisch relevante associaties gevonden tussen een HSV-1 variant en de ziekteverschijnselen in de bestudeerde HK patiënten. De resultaten duiden dat gG of gI varianten niet predisponeren voor een veranderd klinisch beeld van HK.

Hoofdstuk 4 beschrijft de prevalentie en klinische conse-quenties van humane alfa-herpesvirussen in hoornvliesweefsels van hoornvlies donoren en patiënten die een hoornvlies transplantatie (HT) hebben ondergaan. HSV-1 was het meest voortkomende herpesvirus en de hoeveelheid HSV-1 DNA in hoornvlies biopten van getransplanteerde HK patiënten correleerde met de leeftijd van de patiënt, de ziekte-vrije periode, hoornvlies neovascularisatie, pre-HT steroïden behandeling en de ernst van de ziekte. Deze bevindingen pleiten voor de implementatie van real-time HSV-1 PCR op hoornvlies biopten van HK patiënten om de post-HT diagnose en behandeling te verbeteren.

De introductie van acyclovir (ACV) als anti-HSV-1 medicijn heeft de prognose van HK patiënten drastisch verbeterd. Echter, het veelvuldig gebruik van ACV kan leiden tot ACV resistentie (ACV^R), met ernstige gevolgen voor de behandelde patiënt. In **hoofdstuk 5** zijn de prevalentie en genetische eigenschappen van ACV^R HSV-1 hoornvlies kweken in een groot cohort van HK patiënten onderzocht. De resultaten laten een onverwacht hoog percentage aan ACV^R zien: 11 van de 173 onderzochte HSV-1 hoornvlies kweken waren ACV^R. Het merendeel van deze patiënten reageerde niet op ACV therapie. Dit benadrukt het nut van het monitoren van de ACV gevoeligheid van HSV-1 hoornvlies kweken wanneer een klinische ACV^R vermoed wordt. Wanneer ACV^R HSV-1 gevonden wordt, kan foscarnet of cidovovir behandeling gestart worden om de HSV-1-geïnduceerde hoornvlies infectie te controleren.

Hoofdstuk 6 beschrijft het onderzoek aan de incidentie en klinische relevantie van ACV^R in patiënten met recidiverende HK (rHK). Hiervoor werden 38 sequentiële HSV-1 hoorvlies kweken van 15 rHK patiënten in detail bestudeerd. Hoornvlies kweken blijken te bestaan uit zowel ACV sensitieve (ACV^S) als ACV^R varianten van hetzelfde virus. In patiënten die ongevoelig zijn voor ACV therapie werden hoofdzakelijk ACV^R virussen geïsoleerd. De herhaaldelijke isolatie van ACV^R HSV-1 uit hoornvliezen van rHK patiënten geeft aan dat deze ACV^R virussen latent aanwezig kunnen blijven en op een later tijdstip aanleiding kunnen geven tot een klinisch ACV^R recidief. Bij patiënten bekend met een ACV resistente HK wordt aangeraden de ACV gevoeligheid van HSV-1 hoorvlies kweken bij elk recidief zo snel mogelijk te bepalen.

中文摘要 (Chinese Summary)

单纯疱疹病毒（HSV-1）引起的角膜感染称为单纯疱疹病毒性角膜炎(HK)，它的转归取决于 3 种相互作用的因素，即宿主固有免疫的基因组成、适应性免疫、病毒的种属。HSV-1 感染性角膜上皮炎（HEK）主要由病毒感染所造成的细胞坏死、溶解、脱落所致，大多数患者可在短时间内自行消退。而 HSV-1 感染引起的免疫性角膜基质炎（HSK）则是一种由病毒感染所诱导的免疫性疾病，其发病率、致盲率均居各种非创伤性眼科疾病之首，是当今世界上危害最严重的感染性眼病之一。

由于人粒细胞巨噬细胞集落刺激因子（GM-CSF）在慢性炎症性疾病（如类风湿，哮喘）的致病机理中占有重要位置，在本论文的第二章中我们研究并讨论了其在 HSK 的免疫病理学中所起到的作用。人眼角膜细胞可以在 T 细胞和巨噬细胞所分泌的细胞因子的刺激下产生大量 GM-CSF，可以延长多形核白细胞的寿命并使之得到活化。本研究证明，局部减弱或消除 GM-CSF 的作用将会给 HSK 病人的治疗带来积极的作用。

先前的小鼠研究显示，用不同的 HSV-1 病毒株感染小鼠可以诱发不同程度 HK。本论文的第 3 章对于从 HK 患者眼角膜所提取的 HSV-1 样本进行了基因型分析，以找到不同的 gG 和 gI 基因型与不同 HK 临床表征之间的关系。178 个样本的数据显示，HSV-1 的 gG 和 gI 基因型与 HK 的临床表征之间并无显著关联。

在第四章中，我们对在穿透性角膜移植术（PKP）中被移除角膜的 α 疱疹病毒检出率及病人术后的临床表征进行了分析。在 HSV-1，HSV-2 和 VZV 三种 α 疱疹病毒中，HSV-1 的检出率最高。HSV-1 在被移除角膜中的滴度与角膜的无复发生存期，角膜新生血管的形成，以及 HK 的临床表征等紧密相关。这些数据显示，通过实时聚合酶链式反应检测 HK 病人被移除角膜的 HSV-1 滴度将会提高 HK 病人 PKP 后的预后和治疗质量。

阿昔洛韦（ACV）药物的发现在很大程度上提高了 HSV-1 病毒感染所致眼科疾病的治疗效果和预后，但它的广泛应用也不得不面对耐药 HSV-1 病毒株的出现。在第五章中，通过对 173 个 HK 病人样品的耐药性及基因型的特征分析，我们发现 ACV 耐药性的检出率在 HK 病人中是相当高的。因此，长期对那些患有难治性 HK 病人的病毒样本进行耐药性检测是十分必要的。耐药病毒的检测对于病人治疗方法的及时调整将起到至关重要的作用。在第六章中，我们进一步对复发性 HK 病人的连续性样本的耐药性和病人的临床表征进行了分析。通过基因型分析发现，每一个 HK 病人的 HSV-1 样品都是很多病毒株的混合体，它们大都起源于同一祖先，却拥有不同突变的 TK 基因。这个

混合体中也含有耐药病毒株，而整个样本的耐药性则取决于耐药病毒株在此混合体中所占的比例。耐药病毒存同样在于病人的复发样本中，说明这些病毒可以在宿主中建立它们的潜伏状态并复发。这一事实说明，对复发性 HK 病人的病毒样本进行耐药性检测也是同样重要的。

本论文中对 HK 的免疫学致病机理的一些分析，及耐药病毒株的检测手段和分析结果都经对 HK 的诊断及更有效治疗提供了有力的依据。

ABBREVIATIONS

7AAD	7-amino actinomycin D
ACV	Acyclovir
ADCC	Antibody dependent cytotoxicity
APC	Antigen presenting cells
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CI	confidence interval
CM	Conditioned medium
CMV	Cytomegalovirus
CNS	Central nervous system
DMEM	Dulbecco's modified Eagle's medium
DNA	Desoxy ribonucleic acid
DNA pol	DNA polymerase
dpi	days post infection
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopic
FACS	Flow cytometer
FBS	Fetal bovine serum
FOS	Foscarnet
gB	Glycoproteins B
GCV	Ganciclovir
GFP	Green fluorescent protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
HCE	Human corneal epithelial cell
HCF	Human corneal fibroblast
HE	Hematoxylin and Eosin
HEK	Herpes epithelial keratitis
HHV	Human herpes virus
HK	Herpetic keratitis
HLA	Histocompatibility Leukocyte Antigen
HMBS	*Homo sapiens* hydroxyl-methyl-bilane synthase
HPMPC	Cidofovir
HR	hazard ratio
HSV	Herpes simplex virus
HSK	Herpes stromal keratitis
IEK	Infectious epithelial keratitis
Ig	Immunoglobuline
IHC	Immunohistochemistry
IL	Interleukin
INF	Interferon
ISK	Immune stromal keratitis
kb	kilo-base pairs
LAT	Latency-associated transcript
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MHC	Major histocompatibility complex
MOI	Multiplicity of infection
NK cell	Nature killer cell
NSK	Necrotizing stromal keratitis
ORF	Open reading frame
PCR	Polymerase chain reaction
PCV	Panciclovir
PMN	Polymorphonuclear cells
PKP	Penetrating keratoplasty
RE	Restriction enzyme
RFI	Recurrence free interval
RFLP	Restriction fragment length polymorphisms
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute medium
RT-PCR	Reverse transcription polymerase chain reaction
RNAi	RNA interference
SEM	Standard error mean
SFM	Defined keratinocyte serum-free
TGF	Transforming growth factor
TK	Thymidine Kinase
TNF	Tumor necrosis factors
US	Unique short sequence
UL	Unique long sequence
UV	Ultraviolet
VZV	Varicella zoster virus

Curriculum vitae

The author of this thesis was born on August 18th, 1977 in Beijing, China. She obtained her Bachelor of Science degree from China Agricultural University, Beijing, in 1999. In 2000, she studied as a master student of botany at the same university for one year. From 2002, she started the master program of Biotechnology at Wageningen University in the Netherlands, and obtained her master degree in 2004. In the same year she started her Ph.D research at the Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands under the supervision of Prof.dr. A.D.M.E Osterhaus and dr. G.M.G.M Verjans.

Publications

Duan R, de Vries RD, van Dun JM, van Loenen F, Osterhaus AD, Remeijer L, Verjans GM.. Acyclovir Sensitivity and Genetic Characteristics of Sequential HSV-1 Corneal Isolates in Patients with Recurrent Herpetic Keratitis. *Submitted to Journal of Infectious Disease.*

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Patent

Liu JY, Li WJ, Zhao NM, **Duan R**, Fan JH. Rice PHGPx gene，protein and its application. *China Patent, Patent No.: 00109313.4*

PhD Portfolio Summary

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2008	Course on Basic and translational Oncology. One week course in General Oncology provided by the Post-graduate School Molecular Medicine, Erasmus MC and the research school MGC, Leiden.
2007	Symposium Post-infectious Diseases. Two days symposium in post-infectious disease organized by the Post-graduate School Molecular Medicine, Erasmus MC.
2006~2007	Course in English Biomedical Writing and Communication. Five month course provided by the Post-graduate School Molecular Medicine, Erasmus MC.
2005	Course on Laboratory Animal Science. Two weeks training course in laboratory animal science (Article 9 Certificate) provided by Utrecht University.
2004	Course in virology. One-week international training course in General Virology provided by the Post-graduate School Molecular Medicine and the Department of virology, Erasmus MC.
2004	Course in immunology. Two-week international post-doctoral training course in immunology, provided by the Leiden Institute for Immunology.
2004~present	International seminar series in Virology, Immunology, Cell biology, and Molecular Medicine, provided by the Post-graduate School Molecular Medicine and the Department of Virology, Erasmus MC
2004~present	Internal and external presentations at the department of Virology twice a week.