CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO CANDIDA ALBICANS

By

LIM PEI CHING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2005
DEDICATION

To my parents, who put up with me, and

Jin Hoong, who encourage and accompany me always.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO Candida Albicans

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October 2005

Chairman: Professor Seow Heng Fong, PhD

Faculty: Medicine and Health Sciences

Candida albicans is the most common aetiological agent that causes haematogenously disseminated candidiasis. Under conditions that compromise the host immune system, C. albicans disseminates from mucosal sites and results in a progressive disease associated with high rates of mortality. Cytokines are important immunomodulators in coordinating the host defense against C. albicans infection. Human endothelial cells are known to produce various types of cytokines in response to pathogen invasion. The present study was undertaken to identify the cytokines that are involved in the host defense against C. albicans, as well as, to determine the importance of direct cell-to-cell contact in triggering expression of cytokines. In addition, the involvement of Toll-like receptor (TLR)2, TLR4 and nuclear factor-κB (NF-κB) in the host defense against C. albicans were also examined. Expression of cytokines by endothelial cells in response to C. albicans was investigated by using an in vitro model of human umbilical vein endothelial cell line (HUVEC) co-cultured with Candida spp. Both conventional and real time PCR showed that among the cytokines studied, only granulocyte-macrophage colony-stimulating factor (GM-CSF) was found to be differentially expressed in
HUVEC upon stimulation with *C. albicans*. Elevated levels of GM-CSF were found in the co-culture of HUVEC with *C. albicans* but not in the other non-albicans *Candida* spp. Three additional *C. albicans* strains co-cultured with HUVEC also showed a similar pattern of increased GM-CSF expression, although at different levels from strain to strain. This provided evidence that the induction of GM-CSF was not confined to only a particular clinical strain of *C. albicans*. On the other hand, *C. dubliniensis*, which possessed a similar phenotype as *C. albicans* failed to stimulate a similar pattern of GM-CSF expression in HUVEC. The induction of GM-CSF was then found to be contact-dependent via the use of cell culture insert to physically separate *C. albicans* from adhering to the HUVEC monolayer. Pretreatment with anti-TLR2 and anti-TLR4 antibodies showed that TLR4 but not TLR2 was involved in the induction of GM-CSF expression by HUVEC. In addition, pretreatment with SN50 inhibitor also demonstrated that NF-κB may be involved in stimulating expression of GM-CSF transcript. In conclusion, we have discovered that HUVEC is involved in the innate immune response to *C. albicans* by producing GM-CSF cytokine through the activation of TLR4 and also NF-κB transcription factor in a contact-dependent manner.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGZAHIRAN SITOKIN DI DALAM JUJUKAN SEL MANUSIA ENDOTELIUM YANG DIARUH OLEH CANDIDA ALBICANS

Oleh

LIM PEI CHING

Oktober 2005

Pengerusi: Profesor Seow Heng Fong, PhD

Fakulti: Perubatan dan Sains Kesihatan

Candida albicans adalah punca penyakit kandidiasis yang menyebar melalui darah. Apabila sistem pertahanan badan menjadi lemah, C. albicans boleh menyebar melalui mukosa ke dalam organ dalaman dan menyebabkan penyakit kandidiasis menjadi semakin serius dan membawa kepada kadar kematian yang tinggi. Sitokin penting dalam mengkoordinasikan sistem pertahanan untuk melawan jangkitan C. albicans. Jujukan sel manusia endoteliun diketahui boleh menghasilkan pelbagai sitokin untuk melawan serangan patogen. Tujuan penyelidikan ini adalah untuk mengenalpasti sitokin yang terlibat dalam sistem pertahanan yang melawan serangan C. albicans dan untuk mengkaji kepentingan sentuhan dalam jujukan sel manusia endoteliun (HUVEC) dan C. albicans dalam pengzahiran sitokin. Tambahan pula, penglibatan ‘Toll-like receptor’ (TLR)2 dan TLR4 serta Factor nuklear-κB (NF-κB) dalam sistem pertahanan terhadap C. albicans juga dikaji dalam penyelidikan ini. Pengzahiran sitokin dalam HUVEC yang diaruh oleh C. albicans dilakukan melalui penggunaan sebuah model kultur luaran antara HUVEC dengan C. albicans. Kedua-dua teknik tradisional dan “real time” RT-PCR telah menunjukkan di antara sitokin-sitokin yang disiasat, hanya granulocyte-
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First and foremost, I would like to express my heartiest thanks to my supervisor, Prof. Dr. Seow Heng Fong for her invaluable guidance, encouragement and endless support throughout this challenging study. Her constructive criticisms have been crucial in ensuring succeed of this project as well as the writing of this thesis.

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I certify that an Examination Committee met on 28th October 2005 to conduct the final examination of Lim Pei Ching on her Master of Science thesis entitled "Cytokine Production by a Human Endothelial Cell Line in Response to Candida albicans" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

[Signature]

LIM PEI CHING

Date: 17 Jan 2006
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Relative expression of GM-CSF mRNA transcript in HUVEC co-cultured with *C. albicans* 2714 in the absence or presence of respective inhibitors after 4 hours of incubation.
LIST OF ABBREVIATIONS

~
approximately

α
alpha

β
beta

γ
gamma

Δ
delta

κ
kappa

cm
centimeter

g
gram

μg
microgram

pg
picogram

μl
microliter

μm
micrometer

mg
milligram

mM
millimolar

ml
millimeter

nm
nanometer

°C
degree of Celsius

%
percentage

V
volt

bp
base-pair

kb
kilobase-pair
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<tr>
<td>L</td>
<td>liter</td>
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<tr>
<td>ALS</td>
<td>Agglutinin-Like-Sequence</td>
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<td>ECGS</td>
<td>Endothelial Cells Growth Supplement</td>
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<tr>
<td>EDTA</td>
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<td>ELISA</td>
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<td>FBS</td>
<td>Foetal Bovine Serum</td>
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<td>Gly</td>
<td>Glycine</td>
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<td>GM-CSF</td>
<td>Granulocyte-Macrophage Colony-Stimulating Factor</td>
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<td>HBSS</td>
<td>Hank’s Balance Salts Solution</td>
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<td>HUVEC</td>
<td>Human Umbilical Vein Endothelial Cells</td>
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<td>HWP-1</td>
<td>Hyphal wall protein-1</td>
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