GENE EXPRESSION IN DENGUE VIRUS INFECTION

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A thesis submitted

In fulfillment of the requirements for the degree of Doctor of Philosophy

INSTITUTE OF HEALTH AND COMMUNITY MEDICINE
UNIVERSITI MALAYSIA SARAWAK
2008
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Prof Jane Cardosa, for your guidance, advice, and patience throughout these years. Thanks for the knowledge that you have imparted upon me. They’re invaluable. You will always be my inspiration of being a good scientist.

To Ms Tio Phaik Hooi, thank you for your support and assistance throughout my studies. I’ll always remember your generosity.

To all my lovely lab mates and friends, thank you for all your insightful discussion, encouragement, and friendship. You all have made my stay at UNIMAS an enjoyable one! I’ll always remember us as a team.

And, to my parents and siblings for always being there without fail.
Dengue virus infection is a major public health problem worldwide and is amongst the most important human disease caused by mosquito-borne viruses. Despite our growing understanding of the various facets of dengue infection, its pathogenesis still remains elusive. In the first part of this study, we described a real-time PCR method for the detection and quantitation of DENV in the antibody dependent enhancement (ADE) context. ADE has been hypothesized as one of the major known risk factors for the development of DHF. We have demonstrated 2 – 4 fold increase in DENV RNA copy number in antibody mediated enhanced infection in the presence of an enhancing antibody. In the second part of this study, the virus and host interactions at the transcriptional level were investigated. Replication of virus within an infected host cell alters normal cellular gene expression profiles, and triggers immune mediators which might play significant roles in the pathogenesis. Microarray system comprising 40K mouse oligonucleotide cDNA was utilized to study differentially expressed genes following DENV-2 infection of mouse macrophages P388D1 cells. Seven genes that exhibited at least 2 fold up-regulation in expression level were identified. These genes are involved in transcriptional regulation (Scotin, Bst2), dsRNA binding receptor (RIG-I), MHC molecule (B2M), interferon related regulation (IFIT1, Ly6e, Bst2), and growth arrest and apoptosis (Scotin, Mpeg1). Quantitative real-time PCR was then performed to validate the microarray findings and to determine the expression pattern of these gene of interests. Among these, IFIT1 (interferon-induced with tetratricopeptide repeats 1) was the highest up-regulated gene. The gene expression profile of IFIT1 was further analyzed in healthy donors and patients with suspected dengue infections. Our observation showed that IFIT1 is expressed at a basal level in healthy donors and up-regulated during viral infections. Patients with dengue virus infections induced a significant
higher level of IFIT1 than non-dengue patients (p<0.0001), and activation seems to be correlated
with the duration of illness, with expression level found to be higher in the early phase of
infection. This is the first instance where IFIT1 has been shown to be up-regulated in dengue
infection. This suggests that IFIT1 may be important in the pathogenesis of dengue infection.
ABSTRAK

Jangkitan virus denggi merupakan satu masalah kesihatan awam yang meleluasa di serata dunia dan merupakan antara penyakit yang paling penting disebabkan oleh virus yang merebak melalui nyamuk. Walaupun pemahaman tentang pelbagai aspek infeksi virus denggi semakin mendalam, masih terdapat banyak soalan yang tidak terjawab dari segi patogenesis infeksi virus ini. Dalam bahagian pertama kajian ini, kaedah real-time PCR atau reaksi jujukan herantai polymerase semasa telah digunakan untuk mengenalpasti dan mengkuantitasikan virus denggi (DENV) dalam konteks antibody dependent enhancement (ADE) atau penambahan kesan infeksi yang bergantung kepada antibodi. Terdapat satu hipotesis yang mencadangkan ADE sebagai salah satu faktor risiko dalam jangkitan virus denggi yang menjurus kepada demam denggi berdarah (DHF). Kajian ini telah menunjukkan peningkatan RNA DENV sebanyak 2 – 4 kali ganda dalam jangkitan yang pertingkatkan oleh kehadiran satu enhancing antibody atau antibodi penambah. Dalam bahagian kedua kajian ini, interaksi di antara virus denggi dan sel perumah dari segi transkripsi telah dikaji. Replikasi virus dalam sel perumah selepas infeksi virus boleh mengubah profil normal ekspresi gen sel perumah dan mencetuskan perantara-perantara sistem imun yang mungkin terlibat di dalam patogenesis. Sistem microarray yang mengandungi 40K oligonucleotide cDNA tikus telah digunakan untuk mengkaji gen yang diekspresikan berikutan infeksi DENV-2 ke atas sel macrophage tikus P388D1. Tujuh gen yang menunjukkan peningkatan 2 kali ganda dalam tahap ekspresi telah dikenalpasti. Gen-gen tersebut terlibat dalam proses-proses seperti regulasi transkripsi (Scotin, Bst2), reseptor pencantum untuk dsRNA (RIG-I), molekul MHC (B2M), regulasi yang berkaitan dengan interferon (IFIT1, Ly6e, Bst2), serta pembantutan pertumbuhan dan apoptosis (Scotin, Mpeg1). Jujukan berantai polymerase semasa atau real-time PCR yang kuantitatif telah dijalankan untuk
mengesahkan penemuan ujian microarray dan juga untuk menentukan corak ekspresi gen-gen yang mempunyai kepentingan dalam penemuan ini. Di kalangan gen-gen tersebut, IFIT1 (interferon-induced with tetratricopeptide repeats 1) merupakan gen yang mengalami ekspresi yang paling tinggi. Profail ekspresi gen IFIT1 telah dianalisa dengan lebih lanjut dan perbandingan dibuat di kalangan penderma darah yang sihat dengan pesakit yang disyaki dijangkiti denggi. Pemerhatian melalui kajian ini mendapati IFIT1 diekspresikan pada paras yang asas di kalangan penderma darah yang sihat manakala ekspresinya adalah tinggi semasa infeksi virus denggi. Perbezaan paras IFIT1 di kalangan pesakit yang mengalami infeksi virus denggi adalah sangat nyata berbanding dengan bukan pesakit denggi (p<0.0001), dan pengaktifan gen ini mempunyai kaitan dengan jangkamasa penyakit di mana tahap ekspresi gen ini didapati adalah lebih tinggi di peringkat awal infeksi. Penemuan ini merupakan penemuan yang pertama yang penunjukkan bahawa ekspresi IFIT1 meningkat dalam suatu infeksi denggi. Keputusan ini mencadangkan bahawa IFIT1 berkemungkinan mempunyai hubungkait dengan patogenesis infeksi denggi.
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADE</td>
<td>antibody dependent enhancement</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>B2M</td>
<td>beta 2 microglobulin</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>Bst2</td>
<td>bone marrow stromal antigen 2</td>
</tr>
<tr>
<td>C</td>
<td>capsid</td>
</tr>
<tr>
<td>cDNA</td>
<td>complimentary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CF</td>
<td>complement fixation test</td>
</tr>
<tr>
<td>CPE</td>
<td>cytopathic effect</td>
</tr>
<tr>
<td>DENV</td>
<td>dengue virus</td>
</tr>
<tr>
<td>DENV-1</td>
<td>dengue virus serotype 1</td>
</tr>
<tr>
<td>DENV-2</td>
<td>dengue virus serotype 2</td>
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<tr>
<td>DENV-3</td>
<td>dengue virus serotype 3</td>
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<tr>
<td>DENV-4</td>
<td>dengue virus serotype 4</td>
</tr>
<tr>
<td>DF</td>
<td>dengue fever</td>
</tr>
<tr>
<td>DHF</td>
<td>dengue haemorrhagic fever</td>
</tr>
<tr>
<td>DSS</td>
<td>dengue shock syndrome</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>E</td>
<td>envelope</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>fCyR</td>
<td>Fc gamma receptor</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>GAC-ELISA</td>
<td>IgG-capture enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>HI</td>
<td>heat-inactivated</td>
</tr>
<tr>
<td>HK genes</td>
<td>housekeeping genes</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IFIT1</td>
<td>interferon induced protein with tetratricopeptide repeats 1</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IgG</td>
<td>immunoglobulin gamma</td>
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<td>kb</td>
<td>kilobase</td>
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<td>L-15</td>
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<td>LPS</td>
<td>lipopolysaccharide</td>
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<tr>
<td>Ly6e</td>
<td>Lymphocyte antigen Ly-6E precursor</td>
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<tr>
<td>M</td>
<td>membrane</td>
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<tr>
<td>Mab</td>
<td>monoclonal antibody</td>
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<tr>
<td>MAC-ELISA</td>
<td>IgM-capture enzyme-linked immunosorbent assay</td>
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<tr>
<td>MALDI-ToF</td>
<td>matrix-assisted laser desorption-ionization time-of-flight</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MMLV</td>
<td>Moloney murine leukaemia virus</td>
</tr>
<tr>
<td>MOI</td>
<td>multiplicity of infection</td>
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</tbody>
</table>
Chapter 1: Literature review

1.1 Background

The global prevalence of dengue has grown dramatically in recent decades. The disease is now endemic in more than 100 countries in Africa, the Americas, Eastern Mediterranean, Western Pacific and particularly in South East Asia. Distribution of dengue epidemics in the world in the year 2005 is illustrated in Figure 1.1. The World Health Organization (WHO) estimates that more than 2.5 billion people are at risk of dengue infections with 50 - 100 million cases occurring annually. Among these infections, approximately 500,000 cases are dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), with 24,000 deaths which mostly occurred in children (Rigau-Perez et al., 1998; WHO, 2000).

The incidence of dengue virus infections is much greater in Asian countries than in other regions. Outbreaks of dengue haemorrhagic fever have been reported in Indonesia (Sukri et al., 2003), Myanmar (Thu et al., 2004), Thailand (Kittigul et al., 2003; Tuntaprasart et al., 2003), Singapore (Goh et al., 1987), Vietnam, Cambodia, India and Sri Lanka (Pinheiro and Corber, 1997). Dengue fever has also been known to be endemic in Malaysia. Dengue fever (DF) was first reported in Malaysia in 1902 whereas DHF was first reported in 1962 (George, 1992). The first major outbreak occurred in 1973 (George, 1992). Since then, epidemics of dengue cases have been reported regularly. According to Malaysian Ministry of Health’s Disease Control Division, there was a total of 30,285 dengue cases and 65 deaths recorded in the first seven months in the year 2007, compared to 20,258 cases and 49 deaths reported for the same period in year 2006 (http://www.alertnet.org/thenews/newsdesk/K1R72025.htm, 27Sept2007). One of the most important reasons for the increase in cases is
most likely due to rapid development and urbanization, which provide breeding sites for *Aedes aegypti*, the principal mosquito vector responsible for transmission of dengue virus (DENV). Therefore, the emerging pattern and the increasing trend in the incidence of dengue infections is of great concern as there is no specific therapy and a licensed vaccine is not available yet.

**Figure 1.1** Distributions of dengue epidemics in the world in the year 2005. Red: areas where dengue epidemics are reported. Orange: areas where presence of *Aedes aegypti* are confirmed. (Adapted from http://axisoflogic.com/artman/publish/article_25106.shtml, 15Feb2008)