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| 審査研究科 | 人間総合科学研究科 |
| 学位論文題目 | Analysis of genotype-specific and genotype cross-reactive neutralizing antibody response to dengue virus infection in a common marmoset *(Callithrix jacchus)* model (デングウイルス感染における遺伝子型特異的及び遺伝子型交叉性抗体応答のマーモセットモデルを用いた解析) |
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## 論文の内容の要旨
## Abstract of thesis

（目的 Purpose）

Neutralizing antibodies play an important role in protection against dengue virus (DENV) infection. Current tetravalent dengue vaccine against all four serotypes of DENV was developed using only single genotype from each serotype, with the assumption that infection with one genotype within the same serotype will induce protection against all genotypes within the same serotype. It is widely believed that despite antigenic differences, neutralizing antibody epitopes are conserved among the strains within the same serotypes and strain variation does not affect the ability of neutralizing antibodies to confer protection. Nonetheless, the efficacy of tetravalent vaccine is varied by infected virus, and the efficacy of DENV-2 vaccine is the lowest among four serotypes. Studies on the levels of genotype-specific and genotype cross-reactive neutralizing antibodies to genotypes of DENV are still limited. In addition, studies on correlation between antigenic differences in genotypes and levels of viremia and cell-mediated immunity during DENV infection are limited as well.

In the present study, the author (i) determined the levels of genotype-specific and genotype cross-reactive neutralizing antibodies in response to DENV-2 in primary, secondary, and tertiary DENV infections in common marmosets (Callithrix jacchus) model; (ii) determined levels of viremia and immune-related genes upon inoculation with different genotypes and serotypes of DENV in primary,

secondary, and tertiary infections in common marmosets (Callithrix jacchus) model.

(対象と方法 Materials and Methods)

In study (i), the author obtained a total of 59 plasma samples from 34 common marmosets that were either inoculated with clinically-isolated virus strains (N=26), or inoculated with candidate vaccine strain (N=8). Plasma samples were obtained from marmosets after primary, secondary, or tertiary infection of DENV. There were 12 groups of marmosets that were inoculated with clinically-isolated virus strains and 5 groups of marmosets that were inoculated with candidate vaccine strains. The levels of neutralizing antibodies to DENV-2 Cosmopolitan, Asian I, and Asian/American genotypes and DENV-1 were determined using a conventional plaque reduction neutralization assay on day 0 (prior to virus inoculation), 4, 7, and 14 post-inoculations. Student's t-test was used to determine the differences in mean titers of neutralizing antibody between genotypes of each group.

In study (ii), the author collected a total of 19 plasma and peripheral blood mononuclear cells samples on days 0 (prior to virus inoculation), 2, 4, 7, 10, and 14 post- inoculations from 7 groups of common marmosets. There were 2 groups of marmosets for primary infection (N=6), 3 groups of marmosets for secondary infection (N=7), and 2 groups of marmosets for tertiary infection (N=6). Viral RNA copy numbers and infectious virus titers were determined using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and conventional plaque assay, respectively. Levels of immune-related genes including CD3ε, CD4, CD8α, IFNγ, IL-1β, IL-2, and IL-10 were determined using qRT-PCR. Expression levels of each gene were analyzed using comparative $C_T$ method and normalized with levels of GAPDH as reference gene. Differences in levels of immune-related gene on days 2, 4, 7, 10, and 14 p.i were compared with those on day 0 as a reference using Student's t-test.

(結果 Results)

Neutralizing antibodies induced during primary DENV-2 infection neutralized all three genotypes of DENV-2 (Cosmopolitan, Asian I, and Asian/American genotypes). In secondary infection with homologous genotype, levels of genotype-specific and genotype cross-reactive neutralizing antibodies to all three genotypes of DENV-2 did not differ from days 0-14 post-inoculation in marmosets that were inoculated with DENV-2 Cosmopolitan and Asian I genotypes but not in marmosets that were inoculated with DENV-2 Asian/American genotype. Higher levels of neutralizing antibodies to Asian/American genotype than those to Cosmopolitan and Asian I genotypes were observed in secondary infection with heterologous genotype and in tertiary infection. Single infection with DENV-1 did not induce serotype cross-reactive neutralizing antibodies to DENV-2, and following secondary DENV-1 infection, serotype cross- reactive neutralizing antibodies to DENV-2 were induced. Levels of neutralizing antibodies in marmosets that were inoculated with candidate vaccine strain were comparable as those marmosets that were inoculated with clinically isolated parent strain.

Levels of viremia in primary and tertiary DENV infection were different upon inoculation with different genotypes of DENV. In secondary infection, viremia was detected in marmosets that were inoculated with heterologous serotype but absent in those that were inoculated with homologous serotype. Levels of CD3ε, CD4, IFNγ, IL-2, and IL-10 in all groups of marmosets differed. Interestingly, levels of CD8α decreased in early phase of viremia and increased in late phase of viremia. Levels of IL-1β decreased in all marmosets that developed viremia following DENV infection.

(考察 Discussion)

Neutralizing antibodies induced during primary infection were genotype cross- reactive but serotype-specific. Differences in levels of neutralizing antibodies during heterologous infection suggest

heterogeneity in antigenic molecules among genotypes may lead to the induction of higher levels of neutralizing antibodies. Sequential infection induced broad cross-reactive neutralizing antibodies that neutralized multiple serotypes including non-infecting serotypes. Heterogeneity and homogeneity of infecting genotype influence the levels and cross-reactivity of neutralizing antibodies that were induced in following infections. Study on candidate vaccine strain suggested that while attenuation decreased the virus pathogenicity, immunogenicity patterns between genotypes retained. Differences in the levels of viremia may have been caused by antigenic differences. Nonetheless, the correlation between antigenic differences in genotypes of DENV and levels of immune-related genes remained inconclusive. Levels of CD8α inversely correlated with viremia levels, because CD8+ T cells may have been activated to control the viral replication. Decreased levels of IL-1β in marmosets maybe associated with the absence of vascular permeability symptoms.

(結論 Conclusion)
Levels and patterns of genotype-specific and genotype cross–reactive neutralizing antibodies, and viremia levels were different in primary, secondary and tertiary DENV infection in common marmosets. Thus, the author concluded that identification of DENV strains that enable to induce similar levels of neutralizing antibodies and offer protection against homologous or heterologous genotypes of DENV should be addressed in DENV vaccine development study.

## 審査の要旨
## Abstract of assessment result

【批評 General Comments】
Studies on the levels of genotype-specific and genotype cross-reactive neutralizing antibodies to DENV have been limited. This study clarified that heterogeneity and homogeneity of subsequently infected genotypes influenced the levels and cross-reactivity of neutralizing antibodies. Proposed marmoset model will provide a framework for the assessment of neutralizing antibodies against homologous or heterologous genotypes of DENV, which should be addressed in vaccine development studies.

【最終試験の結果 Assessment Result】
The final examination committee conducted a meeting as a final examination on June 11, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】
The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.