学位論文内容の要旨
（Summary of Dissertation）

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学位論文題名
（Dissertation Title）

Gold-nanocluster-mediated microinflammation detection using a tissue clearing method and Molecular mechanism for SARS-CoV-2-specific $CD4^+$ T cell activation in healthy people

(組織透明化法を用いた金ナノクラスターによる微小炎症検出と健常人における SARS-CoV-2 特異的 $CD4^+$ T 細胞活性化の分子機構に関する研究)

Summary

Background and objectives:
Inflammation is a part of biological homeostatic response triggered by an entry of foreign substances into the body, such as pathogens, damaged cells, or irritants, and is a series of biochemical events by mobilizing local immune system, vascular system and various cells within damaged tissues. Upon inflammation, immune system can form a sophisticated immune memory mechanism, which eliminates the previously-encountered infectious agents quickly and strongly. On the other hand, when inflammation is prolonged, chronic inflammation such as autoimmune diseases develop, the state of cells present at the site of inflammation alters, resulting in permanent tissue destruction.
Using mouse models of inflammatory diseases and clinical specimens, my laboratory has previously shown that stimulation of Toll-like receptor ligands, TNF-α, IL-1 or growth factors in tissue-specific non-immune cells causes simultaneous activation of NF-κB and STAT3 in concert with IL-6, and lead to excessive and sustained activation of NF-κB, so called “IL-6 amplifier”, resulting in various chronic inflammatory diseases including autoimmune diseases. More recently, we reported he hypothesis that novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection may be lethal via IL-6 amplifier, in which the induction of cytokine storms may lead to severe disease. Thus, understanding of inflammatory mechanisms will lead to development of effective vaccines for prevention and treatment against emerging infectious and other diseases, and of methods to detect microinflammation as a cause of chronic inflammatory diseases.
In my doctoral research projects, I aimed at (1) exploring and establishing methodology to examine the reactivity and immunodominance of immune memory $CD4^+$ T-cells against viral antigens, which could contribute to the future development of highly effective vaccines against emerging infectious diseases, using COVID-19 as a subject; (2) developing the detection method of microinflammation as a potential cause of chronic inflammatory diseases, in 3D-image with gold nanoclusters and tissue-clearing method in active and pathogenic CD4+ T cells-transfer model of experimental autoimmune encephalomyelitis (EAE; multiple sclerosis model).

Materials and methods:
(1) Twenty-four histidine (His)-tagged SARS-CoV-2 recombinant proteins, including non-structural (NSPs) and structural (SPs) proteins, were synthesized in cell-free system, purified, and bound to Dynabeads. To first determine an optimal incubation time for in vitro study of CD4+ T-cell reactivity and immunodominance, peripheral blood mononuclear cells (PBMCs) from people recovered from COVID-19 were cultured for 4 or 6 days in the presence of Dynabeads-bound viral antigens. The population of activated $CD4^+$ T-cells in the culture

was then assessed. Next, to actually investigate the reactivity and immunodominance of $CD4^+$ T-cells in healthy individuals, PBMCs were cultured for 6 days in the presence of viral antigens and the population of activated $CD4^+$ T-cells was assessed.
(2) Mice induced EAE were treated with gold nanoclusters, and the 1st to 6th lumbar spinal cords (L1-6) were fixed, delipidized, stained with blood vessel markers, and observed under a light-sheet microscope.
Results:
(1) SARS-CoV-2 proteins were successfully synthesized and purified using a cell-free protein synthesis system. The optimal incubation time to examining $CD4^+$ T-cell reactivity and immunodominance to viral proteins was 6 hrs. When PBMCs in healthy individuals were reactivated viral proteins under this culture condition, $CD4^+$ T-cells were more reactive to NSPs than to SPs.
(2) Gold nanocluster particles administered to EAE mice were detected around the dorsal vessels of the L5, which were observed similarly in both active and T-cell-transferred-models of EAE.
Discussion:
(1) The present study supports the previous finding by others that memory T-cells exhibiting cross-reactivity between SARS-CoV-1, SARS-CoV-2 and HuCoV (a common cold coronavirus), exist in SARS-CoV-2-uninfected individuals. In this study, we found that the pre-existing memory $CD4^+$ T-cells in healthy donors reactive to viral NSPs are more frequently than those to SPs, probably being attributed to the high homology of the NSPs from SARS-CoV-2 to those in HCoVs. My study suggests that NSPs may be more useful target than SPs to develop effective vaccines against SARS-CoV-2.
(2) In inflammatory diseases, single-cell events ultimately affect the health of whole organism. Although it has been desired to establish a detection system which enables rapidly to identify the occurrence and progression of microinflammation, that contributes to the pathogenesis of inflammatory disease, conventional detection methods of inflammation have mainly involved 2D- tissue analysis using reporter mice and antibodies for inflammation-related molecules. Through my study, I was able to establish a method to rapidly visualize inflammatory organs in 3D-image, and to corroborate our previous findings that microinflammation at L5 triggers the onset of EAE.
Conclusion:
The immunodominance search and microinflammation detection methods established in my study will further contribute to the establishment of preventive and therapeutic methods for various inflammatory diseases in the future.