A Study of the Activity Control of T-cells by Irradiation with Atmospheric Oxygen Plasma

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（大気圧酸素プラズマ照射によるＴ細胞の活性制御に関する研究）
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論 文 内 容 の 要 旨

Thesis Summary

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Plasma at atmospheric pressure has been intensively studied for potential medical applications, including bacterial sterilization, dermatological interventions, improvement of adaptive immunity, and cancer immunotherapy. Atmospheric pressure plasmas are the most efficient and capable method of generating a large number of active molecules. Plasma irradiation in medical applications directs the active molecules generated from discharge streamers to cells or tissues. Plasma sterilization proceeds in a highly regulated manner by very short time reactions in cells. On the other hand, there is the potential to enhance activity of cells and proteins in the cells. Several studies have shown that plasma irradiation in cancer therapy is one of the most promising medical treatments that can trigger a cancer-specific immune response.

Malaria is a severe infectious disease of humans caused by the plasmodium parasite and is a primary global health problem responsible for the death of over a million people every year. Cerebral malaria is a life-threatening complication of the disease by the parasite Plasmodium falciparum; this complication is characterized by parasite-infected red blood cells accumulating in the brain that induce an immune response during inflammation, such as T lymphocytes (helper T cell). Several previous studies have reported that immune responses to malaria are linked to helper CD4+ T-cells that appear sequentially during the primary infection as a humoral and cellular immune response. Helper CD4+ T-cells reactive to plasmodium infection in red blood cells indirectly stimulate immune cell proliferation and possibly increase proteins that may orchestrate secreted antibody responses or induce the engulfing and absorbing of parasites. However, many studies show it will take about three weeks to obtain the desired cell number in Plasmodium infection in red blood cells. Therefore, it is necessary to examine the activation of antibody-helper CD4+ T lymphocytes, i.e., T helper 1 (Th1) and T helper 2 (Th2) cells, that appear sequentially during primary infection. Th1/Th2 cells produce interferon-gamma (IFN-γ) that plays a role in controlling acute infection, and also produce IL-4 as a humoral immune response, which assists in the maturation of B cells into plasma cells. In this study, we experimentally investigated the activation of two types of T lymphocytes, i.e., EL4 T-cells and BW5147 T -cells sensitized with activator-CD3/CD28 and irradiated with atmospheric oxygen plasma to evaluate the respective and combined effects of sensitization and plasma irradiation on cell activation. This thesis summarizes the research result to solve the above problems, and consists of six chapters as shown below.

In Chapter 1, the background of the above research and the purpose of the research were described. This chapter explained previous studies that short-time irradiation atmospheric pressure plasma has the potential for activating (proliferating, improving function) of the cells. In this study, reactive oxygen species in atmospheric pressure DBD plasma were allowed to act on EL4 and BW5147 T-cells, which are T Lymphocyte Cell Lines. The number of cells was measured using cell survival reagents, and the number of cytokines produced, such as IFN-γ an interferon signal transduction agent, was controlled by the enzyme-linked immunosorbent assay (ELISA) method. In addition, RNA inheritance was extracted from plasma-treated T-cells, and gene expression analysis was performed to confirm changes in T-cell activity.

Chapter 2 described the current status and issues of medical application research on atmospheric pressure plasma. It was also explained about malaria, especially cerebral malaria, as well as current countermeasures and treatment methods.

Chapter 3 described the experimental apparatus and materials used in this research and describes the experiment apparatus and cell culture preparation. First, the structure and configuration of the atmospheric pressure plasma device using the torch-type dielectric barrier discharge (DBD) and the characteristics, i.e., gas temperature, species, and amount of active oxygen produced by atmospheric oxygen plasma, were explained. Next, it was introduced the essential characteristics in the immune system of the two subsets of T Lymphocyte (EL4 T-cells from lymph and BW5147 T-cells from thymus) used in the experiment including their culture method and cell preparation on a microwell plate. Then the method for measuring the number of live/dead cells using a reagent and a cell counter is explained, and as well method for measuring the amount of IFN-γ by the ELISA method were described in detail. The gene expression analysis by the microarray method is also introduced.

Chapter 4 described the activation of T-cells by atmospheric pressure oxygen plasma, i.e., the proliferation and cytokine production characteristics of EL4 T-cells pre-sensitized with a T-cell activation reagent (anti CD3/CD28) then irradiated with various discharge voltages from 4.2 to 6.0 kV and the irradiation period from 0 (i.e., no irradiation) to 40 second. The number of cells increased at specific irradiation time with discharge voltage 4.2 and 5.1 kV. It is the impact of OH radicals and other active oxygen, which is a quantity related to the energy and number density generated by reactive oxygen species when discharge voltage was set to 4.2 or 5.1 kV. Moreover, when the applied discharge voltage at 5.1 kV, the increases of cells different the irradiation period and number of cells is greater than the discharge voltage at 4.2 kV. The increases the number of cells from 10 seconds to 40 seconds (fivefold increase each irradiation period), and after 40 seconds, the number of cells tends to decrease. In this case, although cells grow as an impact of active oxygen, high concentrations of ozone can damage T-cells. In this work, it was also revealed that the production of IFN-γ, which is one of the cytokines that improve the activity of other immune cells, also increases. However, the cell number and IFN-γ production amount show an inverse correlation with the dependence on the discharge voltage. The IFN-γ production amount may fluctuate to cover the increase and decrease in the cell number. The above results are summarized as reference papers 1 and 2 (partial).

Next, we described changes in gene expression levels of the T-cells concerning the production of IFN-γ and cytokines such as Interleukins (IL), which are important mediators for the differentiation of immune cells. In gene expression levels related to the cytokines, the gene expression levels of Nfil3 and Irf4, which are required for T-cell activation, were increased at all discharge voltages of 4.2, 5.1, and 6.0 kV. While the gene expression level of IL-21 decreased an applied discharge voltage at 4.2 kV, the gene expression level of IL-6, IL-16, and IL-4 increased, leading to activation of other cells or T-cells themselves. This result implies that T-cells irradiated by atmospheric oxygen plasma are differentiated to the Th-2 cells. When applied to discharge voltage at 5.1 kV, the gene expression level of IFN-γ, a cytokine peculiar to T-cells, increased. The gene expression level of IL-10, which leads to inhibition of IL-6 and Th-1 cell activation, decreased. Therefore, EL4 T-cells also differentiate into Th-1 cells and produce IFN-γ. It is concluded that T-cells irradiated with the oxygen plasma differentiate into a different cell, i.e., Th-1 and Th-2, depending on the discharge voltage of the atmospheric oxygen plasma. The above results are summarized in Reference Paper 2.

Chapter 5 described the results of investigating the differentiation characteristics of T-cells by plasma irradiation using two subsets of T-cells (EL4 and BW5147 T-cells) derived from lymph and thymus, respectively. Results obtained with a discharge voltage of 4.2 kV increased the number of both T-cells to about 1.5 to 1.8. Moreover, for EL4 T-cells derived from lymph applied with discharge voltage at 4.2 kV, the production of IFN-γ improves with the irradiation time as described above. On the other hand, BW5147 T-cells derived from the thymus Irradiated by plasma with discharge voltage at 4.2 kV obtained that the production of IFN-γ was almost the same as the control (unirradiated) even the plasma irradiation time was changed. From the above, it is considered that T-cells that are sensitized by the time they reach the lymphatic vessels and become CD4+/CD8- Helper T-cells differentiate into Th-1 cells by plasma stimulation and produce IFN-γ. On the other hand, when plasma is applied to CD4+ / CD8+ or CD4- / CD8- T-cells present in the thymus and are not sensitized, they are thought to differentiate into Th-2 cells do not produce IFN-γ. The results proved that T-cells' function could be controlled by selecting T-cell differentiation and irradiating with plasma. From the results of this study, it is thought that IFN-γ production of T-cells can be controlled, leading to the cure and prevention of cerebral malaria. The above results are summarized in Reference Paper 3.

Chapter 6 summarizes this thesis and describes future issues.