学位論文（要約）

Studies on functional regulation of human dendritic cells by IL-6/STAT3 signaling pathway and the effects of anti-tumor immunity in a tumor microenvironment.

(IL-6/STAT3 シグナル経路によるヒト樹状細胞の機能制御と
抗腫瘍免疫への影響に関する研究**)**

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Introduction

Since the discovery of cancer-related antigens in the 1990s, cancer immunotherapy has developed as no side-effect therapy based on cancer-specific activation of the host immune system. To date, several clinical trials of cancer peptide vaccine therapies and cell therapies have been conducted for the treatment of cancer patients. Although some trials reported clinical efficacy, cancer immunotherapy is not a standard therapy for cancer patients. For the development of more effective cancer immunotherapies, it is important to overcome dysfunctional immune responses in the tumor microenvironment. Various immunosuppressive cytokines, such as IL-10 and TGF-β, are produced at high levels in tumor-bearing tissues, and block the function of antitumor effector T cells[5]. In addition, some immune suppressive cells such as $Foxp3^{+}CD4^{+}$ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), induced from immature myeloid cells in tumor microenvironments, are well known to block antitumor immunity **(Figure 1)**.

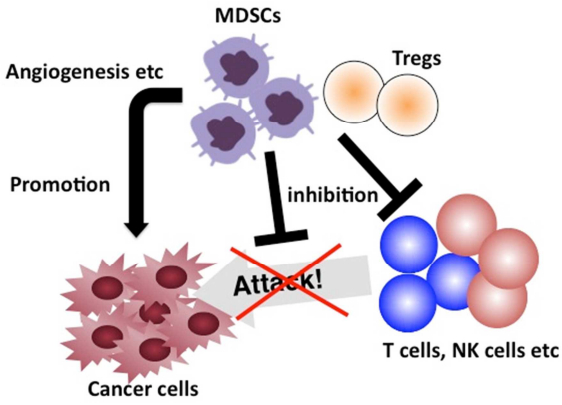

Figure 1. Schema of immunosuppression in the tumor microenvironment.

Recently, effective immuno-check point therapy using anti-PD-1, PD-L1, and/or CTLA-4 antibodies to activate effector T cells in cancer patients was reported in various solid tumor. These results indicate that cancer antigen-specific T cells, which eliminate cancer cells, potentially exist in tumor microenvironments. As a result, blocking of negative signals to tumor-infiltrating T cells can restore the cytotoxic function against the target cancer cells. Thus, introduction of cancer antigen-specific T cells to the tumor microenvironment is required for the first step towards more effective cancer immunotherapy.

Dendritic cells (DCs), representative antigen-presenting cells, strongly induce antigen-specific immune responses through activation of $CD4^+$ T and $CD8^+$ T cells. In cancer patients, DCs expressing HLA-class I, class II, and co-stimulatory molecules on the cell surface are crucial for inducing cancer-related antigen-specific Th cells and cytotoxic T lymphocytes (CTLs) through T cell-DC interaction. Therefore, proper regulation of DC function in tumor-bearing hosts is important for inducing anti-tumor immunity.

IL-6, a pleiotropic cytokine with a variety of effects on cells and tissues, is produced by many different cells including immune cells, fibroblasts, endothelial cells, and tumor cells. IL-6 first binds to the IL-6 receptor (IL-6R); this IL-6/IL-6R complex then associates with the signal-transducing membrane protein, gp130, inducing its dimerization to initiate IL-6 signaling. Gp130 dimerization is followed by the rapid activation of the Janus Kinase (JAK) family and several signaling pathways, including

PI3K/ERK/MAPK and STAT3. STAT3 activation induces numerous effector genes involved in cell proliferation, differentiation and survival.

Previously, it was reported that IL-6 signaling suppresses MHC class II expression on murine DCs via STAT3 activation and attenuates $CD4^+$ T cell-mediated immune responses **(Figure 2)**.

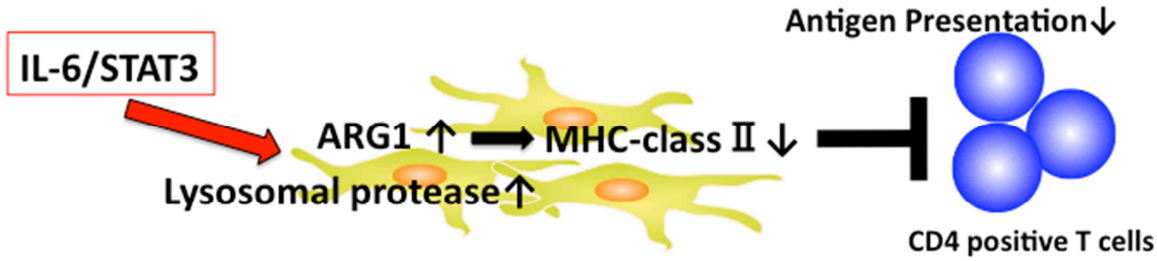

Figure 2. IL-6/STAT3 signaling attenuates the antigen-presenting function of murine DCs via arginase or lysosomal protease activities.

Furthermore, the previous study indicated that administration of monoclonal antibody antagonizing IL-6R (anti-IL-6R mAb) enhanced T cell responses and inhibited tumor growth *in vivo*. In tumor-bearing mice, IL-6 suppressed $CD4^+$ T cell-mediated immunity through down-regulation of MHC class II by enhanced arginase activity of DCs. Thus, IL-6-mediated STAT3 activation appears to be a critical mechanism for inducing dysfunctional immune system responses in the tumor microenvironment via regulation of antigen-presenting cells. Blockade of IL-6/STAT3 signaling cascades may therefore be a promising approach to overcome the dysfunction of antitumor immunity in cancer patients. However, the precise effects of IL-6 on human cancer immunology

are not clear.

Therefore, work in this dissection focused on the IL-6/STAT3-signaling pathway in human DCs, aiming to translate the information obtained by murine studies for clinical benefit in human subjects, and demonstrating the possibility of improving cancer immunotherapy by regulation of the IL-6/STAT3 signaling pathway.

Result and Discussion

This study showed that the IL-6/STAT3 signaling pathway impaired the antigen-presenting function of human DCs via downregulation of HLA-class II expression, and attenuated Th1 immunity by decreasing IL-12 production by DCs. In addition, IL-6 inhibited the maturation of $CD11b^{+}CD11c^{+}$ myeloid cells into antigen-presenting cells. It is well known that patients with advanced cancer are immunocompromised, and cancer progression is closely related with chronic inflammation. IL-6 is a representative pro-inflammatory cytokine, and prospective and retrospective studies have shown that serum IL-6 levels are related to tumor stage and size, metastasis and survival in colon cancer patients, chemotherapy efficacy for advanced pancreatic cancer, advanced stage and metastasis-related morbidity of breast cancer, or clinical efficacy of personalized peptide vaccination for advanced biliary tract cancer. These information suggest that IL-6 levels in cancer may not be simply promising prognostic biomarkers but also associated with tumorigenesis and antitumor immune responses. In this study, IL-6-conditioned MoDCs had reduced antigen-presenting function for Th cells. The helper function of antigen-specific Th cells such as production of Th1 cytokine was essential for inducing fully activated CTLs in tumor-bearing hosts. A previous report demonstrated that cancer antigen-derived peptide containing helper epitope could induce CTLs efficiently *in vitro* according to the helper function of antigen-specific $CD4^{+}$ T cells. In fact, cancer peptide vaccine containing helper epitope could induce CTLs in an advanced cancer patient, and the

patient experience clinical benefit. In particular, activation of Th1 cells is crucial for anti-cancer immunity. IL-12 is an important cytokine for inducing Th1 immunity, because IL-12 activates STAT4 in $CD4^+$ T cells, inducing subsequent IFN-γ secretion. This study showed that IL-6/STAT3 signaling attenuated IL-12 production of MoDCs, and actually impaired IFN-γ secretion of $CD4^+$ T cells *in vitro*. Therefore, it may be that the IL-6/STAT3 signaling cascade attenuated anti-cancer immunity according to the impaired Th1 immunity by reduced IL-12 production.

Moreover, IL-6 stimulation also reduced HLA-DR and CD86 expression levels in $CD11b^+CD11c^+$ cells from PBMCs of healthy donors in a STAT3-dependent manner. In addition, $CD11b^+CD11c^+$ cells pretreated with IL-6 were confirmed to impair T cell-stimulating ability. In this study, activation of arginase, lysosome proteases and COX-2 were involved in the downregulation of HLA-DR on $CD11b^+CD11c^+$ cells, and gene expression of ARG1, CTSL and COX2 were increased by IL-6 stimulation. Therefore, arginase activity, lysosomal protease activity, and COX-2 may be effector molecules for downregulation of HLA-DR and CD86 by IL-6 stimulation.

IL-6 is produced by various cells including cancer cells, cancer-associated fibroblasts, and immune cells due to chronic inflammation in the tumor sites of colorectal cancers. Finally, we investigated the effect of IL-6 in the human colorectal cancer tissue. In this study, $CD11b^+CD11c^+$ cells in colorectal tumor tissues reduced the surface expression levels of HLA-DR and CD86 compared with the PBMCs. *ARG1*, *CTSL* and *COX2* levels were increased in tumor-infiltrating $CD11b^+CD11c^+$ cells. In addition, tumor-infiltrating $CD11b^+CD11c^+$ cells impaired T cell-stimulating ability

compared with PBMCs. These data suggest that tumor-infiltrating myeloid cells may downregulate the surface expression of HLA-DR and CD86 by arginase-1, lysosomal protease and COX-2. Thereby, IL-6/STAT3 signaling inhibits the maturation of tumor-infiltrating myeloid cells into antigen-presenting cells, and attenuates the subsequent T cell-stimulating ability.

It was reported that the presence of intra-tumoral T cells was a good prognostic factor in colorectal cancers. In this study, HLA class II expression levels of tumor-infiltrating immune cells were closely related to the invasion of $CD4^+$ T and $CD8^+$ T cells at tumor sites. This information suggested that appropriate and adequate antigen presentation is essential and important for the introduction of cancer antigen-specific T cells into the tumor microenvironment.

The present findings indicate that maintenance of the antigen-presenting ability of DCs and other myeloid cells is critical for the induction and activation of cancer antigen-specific T cells in the tumor microenvironment. Therefore, the therapeutic strategy for improving the function of DCs is effective in increasing good response to cancer immunotherapies such as cancer vaccines.

Therefore, IL-6 may be a promising target for cancer immunotherapy through both improving the antigen-presenting function of DCs and facilitating maturation of antigen-presenting cells into tumor-infiltrating cells.

Summary and Conclusion

1. IL-6-induced STAT3 activation causes attenuation of antigen-presenting function and IL-12 production by human DCs, resulting in the subsequent suppression of Th1 immunity.
2. IL-6/STAT3 signaling pathway inhibits differentiation of antigen presenting cells into human myeloid cells.
3. IL-6/STAT3 signaling is one factor that causes inadequate T cell-mediated anti-tumor immunity via impairment of the antigen-presenting function of myeloid cells including DCs.

IL-6 causes immunosuppression in cancer patients by inducing dysfunction of antigen-presenting cells such as DCs, suggesting that inhibition of the IL-6/STAT3 signaling pathway will be a promising strategy for the development of novel cancer immunotherapy via improvements in antigen presentation to induce anti-tumor effector T cells in the tumor microenvironment **(Figure 3)**.

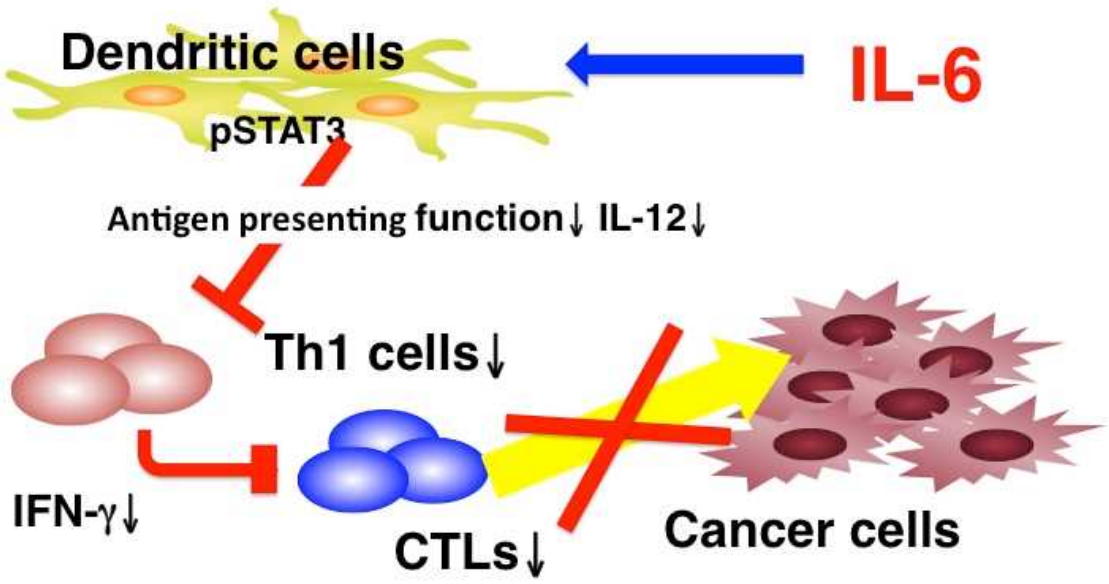

Figure 3. Outline for IL-6/STAT3-mediated dysfunction of dendritic cells in tumor microenvironment.

In this study, information from basic studies using murine models was applied to human subjects. To apply the present findings to the clinic, therapeutic models targeting the IL-6/STAT3 signaling pathway were established using experimental animals because it is necessary to reveal more detailed information about the role of IL-6 in the tumor microenvironment. The effects of the IL-6/STAT3 signaling pathway on antitumor immunity are still being investigated to develop effective cancer immunotherapies.

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