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学　位　論　文（要約）

Studies on biological properties and immunosuppressive function
of myeloid-derived suppressor cells
in tumor microenvironment

（がん微小環境におけるミエロイド由来抑制性細胞群の性状とその
免疫抑制機能に関する研究）

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Th1-dependent immunity is critical for induction of tumor-specific cytotoxic T lymphocytes (CTLs) and memory T cells. However, it is difficult to induce tumor-specific T cell responses in tumor-bearing hosts because of the strong immunosuppressive and tumor-evading mechanisms. Activation of antitumor effectors such as tumor-specific $CD4^+$ T and $CD8^+$ T cells is necessary for suppression of tumor growth but is inhibited by various mechanisms in tumor-bearing state. It is well known that immunosuppressive factors such as interleukin (IL)-6, IL-10, and transforming growth factor (TGF)-β impair the functions of T cells and dendritic cells (DCs) in the tumor microenvironment. Regulatory T cells (Tregs) are a major immunosuppressor of T cell activation in tumor-bearing hosts. Indeed, several strategies that control of Treg-mediated immune suppression has been developed to enhance CTL-mediated antitumor activity in tumor-bearing mice.

In Capter 1, we have revealed that the splenic $CD11b^+Gr\text{-}1^+$ cells, which were called immature myeloid cells (ImC) or myeloid-derived suppressor cells (MDSCs) and abnormally increased in tumor-bearing mice, were classified into three different subsets according to their phenotypic and morphological characteristics; $Gr\text{-}1^{low}$ $F4/80^+$ Macrophage (MΦ-ImC), $Gr\text{-}1^{mid}$ stab cells ($Neut_{stab}$-ImC) and $Gr\text{-}1^{high}$ segmented cells ($Neut_{seg}$-ImC). The splenic MΦ-ImC but not $Neut_{stab}$-ImC and $Neut_{seg}$-ImC exhibited a significant immunosuppressive activity in MLR. In contrast, tumor-infiltrating $Neut_{seg}$-ImC markedly inhibited MLR. Interestingly, we first demonstrated that administration of anti-IL-6R mAb or Gemcitabine (GEM) selectively reduced the MΦ-ImC and $Neut_{stab}$-ImC populations. The elimination of immunosuppressive ImC enhanced $CD8^+$ T cell responses and inhibited the tumor growth. Moreover, co-administration of anti-IL-6R mAb and GEM exhibited higher antitumor effect in comparison to treatment with the single agent.

In Capter 2, we induced $CD11b^+CD14^+$ monocytic MDSCs from peripheral blood mononuclear cells (PBMCs) of healthy donors under the addition of IL-11. The PBMCs treated with IL-11 were highly expressed various immunosuppressive molecules including IL-10 and Arg-1. T-cell proliferation

after the CD3/CD28-stimulation was remarkably reduced when $CD11b^+$ $CD14^+$ cells generated in the presence of IL-11 were co-cultured with autologous $CD4^+$ T or $CD8^+$ T cells. Here, we found that IL-11 stimulation of normal PBMCs led to the STAT3 phosphorylation. In addition, we confirmed that blockade of the IL-11-mediated STAT3 phosphorylation strongly inhibited differentiation of MDSCs. These findings suggest that IL-11 produced under tumor microenvironments may induce monocytic human MDSCs in cancer patients.

These results strongly suggested that both IL-6 and IL-11 regulated the immunosuppressive MDSC populations in tumor-bearing host and would be a promising target in cancer immunotherapy.

CHAPTER 1

Blockade of IL-6 signaling eliminates $CD11b^{+}Gr\text{-}1^{+}$ MDSCs and inhibits the tumor growth by activation of antitumor immunity.

MDSCs are an important negative immunoregulator in tumor-bearing hosts. In mice, heterogeneous populations of $CD11b^{+}Gr\text{-}1^{+}$ cells are classified into different subsets according to their phenotypic and morphological characteristics. In human cancer patients, MDSCs are generally defined as monocytic ($CD11b^{+}$, $CD14^{+}$, and HLA-$DR^{-/low}$) or granulocytic ($CD11b^{+}$ and $CD15^{+}$) myeloid cells with immunosuppressive activities. These cells have been detected in blood of patients with glioblastoma, lung cancer, breast cancer, colon cancer, and melanoma. MDSCs strongly inhibit antitumor T cell responses by numerous factors such as arginase-1, S100A8, S100A9, NADPH oxidase, reactive oxygen species, hydrogen peroxide, and peroxynitrite. In addition, MDSCs have been shown to suppress function of natural killer cells. Although MDSCs are considered to be a potential target for tumor immunotherapy, the characteristics of morphological and functional heterogeneity and responsible factors regulating each subpopulation remain unclear. Therefore, it is important to elucidate the precise immunosuppressive mechanisms of MDSCs in tumor-bearing hosts.

In this study, we revealed that splenic $CD11b^{+}Gr\text{-}1^{+}$ cells were classified into three different subsets: $Gr\text{-}1^{high}$; segmented neutrophil ($Neut_{seg}$-ImC), $Gr\text{-}1^{mid}$; stab neutrophil ($Neut_{stab}$-ImC), $Gr\text{-}1^{low}$; macrophage (MΦ-ImC), in which $Neut_{seg}$-ImC and $Neut_{stab}$-ImC did not suppress T cell responses. In contrast to spleen, tumor-infiltrating $CD11b^{+}Gr\text{-}1^{+}$ cells were consisted in $Gr\text{-}1^{high}$ $Neut_{seg}$-ImC and $Gr\text{-}1^{low}$ MΦ-ImC, which had marked immunosuppressive potency. In patients with various types of cancers, systemic levels of inflammatory cytokines, such as IL-1β, TNF-α, and IL-6 are elevated. Recently, Kishimoto's group has been demonstrated the therapeutic impact of humanized anti-IL-6R mAb on rheumatoid arthritis, Castleman's disease and multiple myeloma. However, antitumor effects on solid tumors and precise mechanisms of antitumor effects were remained

unknown. In this study, we first demonstrated that anti-IL-6R mAb treatment decreased the MDSC populations and augment $CD8^+$ T cell-mediated antitumor immunity in tumor-bearing mice.

We showed in the present study that $CD11b^+Gr\text{-}1^+$ cells, accumulating in the tumor tissues and spleen, contain morphologically-distinct three subsets, which also exhibited distinct immunoregulatory function respectively. Importantly, we demonstrated that two granulocytic cell subsets ($Neut_{seg}$- and $Neut_{stab}$-ImC) in the spleen did not inhibit T cell responses, whereas tumor-infiltrating $Neut_{seg}$-ImC had marked suppressive potency, indicating that all $CD11b^+Gr\text{-}1^+$ cells, commonly-termed MDSCs, were not immunosuppressive myeloid cells and tumor microenvironment was critical condition to obtain inhibitory function.

It is important how blockade of IL-6 signaling decrease MDSC population. We confirmed that when anti-IL-6R mAb was administrated into tumor-bearing mice, the numbers of MDSCs were immediately decreased, suggesting that IL-6 may directly maintain MDSC survival and/or proliferation. Thus, treatment with anti-IL-6R mAb not only eliminates of MDSCs, also modulates MDSC function in the tumor-bearing host. In the tumor-bearing condition, IL-6, produced by tumor cells, macrophages and stromal cells, is involved in immunosuppression through reducing DC function. Moreover, IL-6 directly influences promotion of tumor cell survival and proliferation in a STAT3 dependent manner. These functions of IL-6, coupled with elimination of MDSCs, would also affect antitumor effect of anti-IL-6R mAb treatment.

CHAPTER 2

IL-11-mediated STAT3 activation is required for functional differentiation of myeloid-derived suppressor cells in human.

Activation of ant-tumor effectors such as tumor-specific $CD4^+$ T and $CD8^+$ T cells is necessary for the suppression of tumor growth. However, it is also difficult to induce tumor-specific T cell responses in tumor-bearing hosts, because they are suffering from strong immunosuppressive tumor-escape mechanisms.

MDSCs are a heterogeneous population of immature myeloid cells that generated from the bone marrow and inhibit tumor-specific immune responses. Recently, two distinct subpopulations of MDSCs, $CD11b^+Gr\text{-}1^{high/mid.}$ ($Ly\text{-}6G^+Ly\text{-}6C^{low}$) granulocytic MDSCs and $CD11b^+Gr\text{-}1^{low}Ly\text{-}6G^{-/low}$ $Ly\text{-}6C^{high}$ monocytic MDSCs, have been described. MDSCs exhibit the $CD11b^+Gr\text{-}1^+$ phenotype, and their human counterpart is identified as being $CD11b^+CD14^+HLA\text{-}DR^-$ or $CD11b^+CD15^+$. These MDSCs suppress T cell- and NK cell-responses. MDSCs have been shown to suppress immune responses through a variety of factors, including arginase-1, S100A8, S100A9, inducible nitric oxide synthase (iNOS), and production of reactive oxygen species (ROS). We reported that strategies aimed at the elimination or inhibition of MDSCs by administration of anti-IL-6 mAb and/or Gemcitabine might significantly improve anti-tumor responses and the efficacy of cancer immunotherapy.

IL-11 is a member of IL-6 family, which is defined by the shared use of the gp130, IL-6 receptor β-subunit. IL-11 has a role of systemic inflammation, and promotes platelet production. More recently, IL-11 was reported to be involved in a mechanism of inflammation, angiogenesis and metastasis in tumor microenvironments. IL-11 was produced by cancer-associated fibroblasts and myeloid cells in many types of tumors such as stomach, liver, pancreas, colon, ovary and breast cancer. However, there is no report that the relationship between the suppression of anti-tumor immune response and IL-11-madiated activation of STAT3.

In this study, we found that IL-11-STAT3-signaling cascade regulated functional differentiation of $CD11b^{+}CD14^{+}$MDSCs. Indeed, a specific inhibitor of STAT3 significantly blocked the differentiation of MDSCs induced by IL-11. Moreover, PBMCs from healthy donors acquired immunosuppressive activity, when they were cultured with IL-11. The results suggested that IL-11-induced MDSCs might play a role in immunosuppression of tumor microenvironments. Therefore, regulation of the IL-11-mediated functional differentiation of MDSCs may be a possible target for cancer immunotherapy. Given the critical roles served by MDSCs, our elucidation for the suppressive function of IL-11 has implications in rational design of strategies for modulation of immune responses and development of new efficient treatments for cancer patients.

Immunotherapeutic significance of the discovery and future perspectives

In Capter 1, the limitation of therapeutic efficacy on cancer immunotherapy is reported. Overcoming strong immunosuppression and tumor escape mechanisms in tumor microenvironment is necessary for development of an effective immunotherapy. Therefore, it is important to elucidate the immunosuppressive mechanisms of tumor-bearing hosts for successful treatment of tumors. Myeloid-derived suppressor cells (MDSCs) are one of the major immunosuppressive cell types in tumor microenvironments.

In this study, we first found that the splenic $CD11b^{+}Gr\text{-}1^{+}$ cells were classified into three different subsets according to their phenotypic and morphological characteristics. In contrast to spleen, $CD11b^{+}Gr\text{-}1^{+}$ cells infiltrated into tumor tissues strongly inhibited T cell activation, indicating that $CD11b^{+}Gr\text{-}1^{+}$ cells acquired an immunosuppressive activity under the tumor microenvironments.

Moreover, we found that administration of Gemcitabine (GEM) and/or anti-IL-6 mAb, both of which had regulatory effect on differentiation of MDSCs, into tumor-bearing mice blocked the accumulation of MDSCs at tumor site and inhibited the growth of tumor cells.

Our present results intended to suggest that the combination of chemotherapy with immunotherapy potentially leads to more developed application of effective treatments in the future. Reacenty, it has been reported that the combined treatment with radiation and tumor-specific Th1 cell therapy induced anti-tumor immunity, very efficiently. Therefore, the combination of anti-IL-6R mAb with chemotherapy or cancer vaccine therapy would be possible to enhance the anti-tumor effects. We believe that anti-IL-6R mAb leads to decrease numbers of MDSCs in cancer patients and IL-6 signaling pathway is a good target for regulation of MDSC function during cancer immunotherapy.

Significance of the discovery and future perspectives in the regulation of

MDSCs

In Capter 2, productions of inflammatory cytokines (IL-1β, IL-6, and IL-11) and inductions of MDSCs in the tumor microenvironment are crucial factors limiting the efficacy of immune-based therapy.

In this study, we first found that $CD11b^+$ $CD14^+$ monocytic MDSCs were generated from PBMCs of healthy donors in the presence of IL-11. The IL-11-induced MDSCs upregulated immunosuppressive molecules such as arginase-1. T-cell proliferation was significantly reduced when $CD11b^+CD14^+$ cells, generated in the presence of IL-11, were co-cultured with autologous $CD4^+$ T and $CD8^+$ T cells after stimulation with anti-CD3/CD28 mAbs. IL-11 stimulation induced STAT3 phosphorylation in normal PBMCs. We further confirmed that neutralization of IL-11 inhibited the STAT3 phosphorylation and differentiation of PBMCs into MDSCs. Blockade of STAT3 activation suppressed IL-11-dependent induction of MDSCs. Immunohistochemical analysis indicated that both IL-11 and pSTAT3 was observed at stromal region of tumor tissues from colon cancer patients. These findings suggest that IL-11-STAT3 signaling pathway is a potential target for induction of MDSCs in the tumor microenvironment. Thus, IL-11-STAT3-mediated regulation in functional differentiation of MDSCs will be a promising strategy for improving cancer immunotherapy.