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Unsolicited Review Article

Interleukin-6/STAT3 signaling as a promising target to improve the efficacy of cancer immunotherapy

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Overcoming the immunosuppressive state in tumor microenvironments is a critical issue for improving the efficacy of cancer immunotherapy. Interleukin (IL)-6, a pleiotropic cytokine, is highly produced in the tumor-bearing host. Previous studies have indicated that IL-6 suppresses the antigen presentation ability of dendritic cells (DC) through activation of signal transducer and activator of transcription 3 (STAT3). Thus, we focused on the precise effect of the IL-6/STAT3 signaling cascade on human DC and the subsequent induction of antitumor T cell immune responses. Tumor-infiltrating $CD11b^+CD11c^+$ cells isolated from colorectal cancer tissues showed strong induction of the IL-6 gene, downregulated surface expression of human leukocyte antigen (HLA)-DR, and an attenuated T cell-stimulating ability compared with those from peripheral blood mononuclear cells, suggesting that the tumor microenvironment suppresses antitumor effector cells. *In vitro* experiments revealed that IL-6-mediated STAT3 activation reduced surface expression of HLA-DR on $CD14^+$ monocyte-derived DC. Moreover, we confirmed that cyclooxygenase 2, lysosome protease and arginase activities were involved in the IL-6-mediated downregulation of the surface expression levels of HLA class II on human DC. These findings suggest that IL-6-mediated STAT3 activation in the tumor microenvironment inhibits functional maturation of DC to activate effector T cells, blocking introduction of antitumor immunity in cancers. Therefore, we propose in this review that blockade of the IL-6/STAT3 signaling pathway and target molecules in DC may be a promising strategy to improve the efficacy of immunotherapies for cancer patients.

Since the discovery of cancer-related antigens in the 1990s, cancer immunotherapy has developed as a promising cancer treatment based on cancer-specific activation of the host immune system.[1,2] To date, many basic studies and clinical trials of cancer peptide vaccines and adoptive T cell therapies have been conducted for the treatment of cancer patients.[3,4] However, while some trials have reported clinical efficacy, cancer vaccine therapies are not yet a standard therapy for cancer patients. To develop more effective cancer immunotherapies, it is crucial to induce strong and persistent antitumor immune responses in the tumor microenvironment.

It has been reported that dysfunction of antitumor immunity occurs in tumor-bearing hosts. Various immunosuppressive cytokines, including interleukin (IL)-10 and transforming growth factor (TGF)-β, produced at high levels in tumor-bearing states inhibit the function of antitumor effector T cells. In addition, immune suppressive cells, such as $Foxp3^+CD4^+$ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC), induced from immature myeloid cells in tumor microenvironments are well known to block antitumor immunity.[5–7]

Recently, effective immune checkpoint therapy using anti-programmed cell death protein-1, anti-programmed death-ligand 1 and/or anti-cytotoxic T lymphocyte (CTL)-associated protein-4 antibodies to activate effector T cells in cancer patients has been reported for various solid tumors.[8–10] These results indicate that cancer antigen-specific T cells, which eliminate cancer cells, potentially exist in tumor microenvironments. As a result, blocking negative signals to tumor-infiltrating T cells can restore their cytotoxic function against target cancer cells. Thus, introduction of cancer antigen-specific effector T cells into the tumor microenvironment is required as the first step towards more effective cancer immunotherapy.

Dendritic cells (DC), representative antigen-presenting cells, strongly induce antigen-specific immune responses through

activation of CD4^{+} T and CD8^{+} T cells. In cancer patients, DC expressing human leukocyte antigen (HLA) class I, HLA class II and co-stimulatory molecules on their cell surface are crucial for induction of cancer-related antigen-specific T helper (Th) cells and CTL through the T cell–DC interaction.[11–14] Therefore, proper regulation of DC functions in tumor-bearing hosts is important to induce antitumor immunity.

Interleukin (IL)-6, a pleiotropic cytokine with various effects on cells and tissues, is produced by many different cell types, including immune cells, fibroblasts, endothelial cells and tumor cells.[15,16] IL-6 first binds to the IL-6 receptor (IL-6R). The 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 rapid activation of the Janus kinase (JAK) family and several signaling pathways, including phosphatidylinositol-3-kinase/extracellular signal-regulated kinase/mitogen-activated protein kinase and signal transducer and activator of transcription 3 (STAT3). STAT3 activation induces numerous effector genes involved in cell proliferation, differentiation and survival.

Previously, it was reported that IL-6 signaling suppresses major histocompatibility complex (MHC) class II expression on murine DC through STAT3 activation, and attenuates CD4^{+} T cell-mediated immune responses.[17,18] Furthermore, we found that administration of a monoclonal antibody antagonizing IL-6R enhances T cell responses and inhibits tumor growth *in vivo*.[19,20] Next, we confirmed that IL-6 suppresses CD4^{+} T cell-mediated immunity through downregulation of MHC class II by enhanced arginase activity of DC in tumor-bearing mice. IL-6-mediated STAT3 activation appears to be a critical mechanism for induction of dysfunctional immune system responses in the tumor microenvironment through regulation of antigen-presenting cells. Thus, blockade of IL-6/STAT3 signaling cascades in DC may be a promising approach to overcome the dysfunction of antitumor immunity in cancer patients.

Some studies using murine tumor-bearing models have indicated the positive effects of IL-6 in anti-tumor immunity. A previous paper demonstrated that IL-6 produced by non-hematopoietic stromal cells acted cooperatively with soluble IL-6Rα and thermally induced gp130 to promote E/P-selectin-dependent and ICAM-1-dependent extravasation of cytotoxic T cells in tumors and apoptosis of tumor targets.[21] Another paper revealed that cryo-thermal therapy induced IL-6 at acute phase and shifted the tumor chronic microenvironment from Th2 immunosuppressive and pro-tumorigenic to a Th1 immunostimulatory and tumoricidal state.[22]

Because the precise effects of IL-6 on human cancer immunology are unclear compared with those in mice, we have recently focused on the IL-6/STAT3-signaling pathway in human DC.[23] Our aim was to translate the information obtained by murine studies for clinical benefit in human subjects, and to demonstrate the possibility of improving cancer immunotherapy by regulation of the IL-6/STAT3 signaling pathway.

In this review, we first describe IL-6 production in cancer patients and then the regulatory mechanism of MHC/HLA class II expression in murine and human DC *in vitro*. Then, we discuss the effect of IL-6/STAT3 signaling on the antigen-presenting ability of DC and Th1 immunity in tumor-bearing models. Moreover, we introduce current basic and clinical studies on blocking the effects of the IL-6 signaling cascade on tumor-bearing mouse models and cancer patients. Finally, we propose that proper regulation of the antigen-presenting ability of DC is important for the activation of effector T cells that infiltrate into the tumor microenvironment, and that the IL-6/STAT3 signaling pathway is a promising target to improve cancer immunotherapy.

Interleukin-6 Production in Cancer Patients

Generally, DC immediately activate and initiate T cell immune responses after stimulation by immunological adjuvants such as toll-like receptor (TLR) ligands, whereas in the absence of such maturation signals, most DC remain in an immature form that induces tolerance to self-antigens. Thus, elucidation of the regulating mechanisms of mature and immature statuses is essential for effective vaccine development and prevention of undesirable immune responses by DC in tumor-bearing hosts.

It is well known that patients with advanced cancer are immunocompromised, and cancer progression is closely related to chronic inflammation. IL-6 is a representative proinflammatory cytokine. Prospective and retrospective studies have shown that serum IL-6 levels are related to the tumor stage and size, metastasis and survival of colon cancer patients,[24] chemotherapy efficacy for advanced pancreatic cancer,[25] neoadjuvant chemoradiotherapy for esophageal carcinoma,[26] advanced stage and metastasis-related morbidity of breast cancer,[27] and clinical efficacy of personalized peptide vaccination for advanced biliary tract cancer[28] and colorectal cancers.[29]

Interleukin-6 is produced by various cell types, including cancer cells and cancer-associated fibroblasts because of chronic inflammation in tumor sites.[15,16] We investigated IL-6 production and STAT3 activation in tumor tissues of colorectal cancer patients (Fig. 1), and found strong induction of IL6 gene expression in CD11b^{+}CD11c^{+} myeloid cells of colorectal tumor tissues compared with those from peripheral blood mononuclear cells (PBMC).[23]

Based on this information, we speculate that systemic and local production of IL-6 in cancer patients may be not only useful as promising prognostic biomarkers but also associated with tumorigenesis and antitumor immune responses.

Inhibition of Dendritic Cells Maturation by Interleukin-6 through STAT3 Activation

A previous study has demonstrated that IL-6-deficient mice have increased numbers of mature DC, indicating that IL-6 plays a major role in blocking DC maturation *in vivo*.[17] In this study, STAT3 activation by IL-6 was involved in the suppression of TLR4 ligand and lipopolysaccharide (LPS)-induced DC maturation and activation. Furthermore, DC-mediated helper T and killer T cell responses were remarkably enhanced in IL-6-deficient mice. These findings suggest that IL-6 is a potent negative regulator of DC maturation *in vivo*, and the IL-6/STAT3 signaling cascade in DC may represent a critical target for controlling T cell-mediated immune responses in the tumor-bearing state.

We further demonstrated that the IL-6/STAT3 signaling pathway suppresses MHC class II expression on DC and attenuates CD4^{+} Th cell responses through activation of lysosomal protease in a mouse model.[18] Inhibitors of lysosomal proteases blocked the reduction of MHC class II αβ-dimer in IL-6-treated DC. In addition, gene overexpression of cathepsin S, a lysosome protease, in DC decreased intracellular MHC class II αβ-dimer levels, LPS-mediated surface expression of MHC class II, and suppressed CD4^{+} T cell activation. These data indicate that IL-6/STAT3-induced excessive activation of lysosomal proteases suppresses the functions of DC through the degradation of MHC class IIαβ-dimer in lysosome.
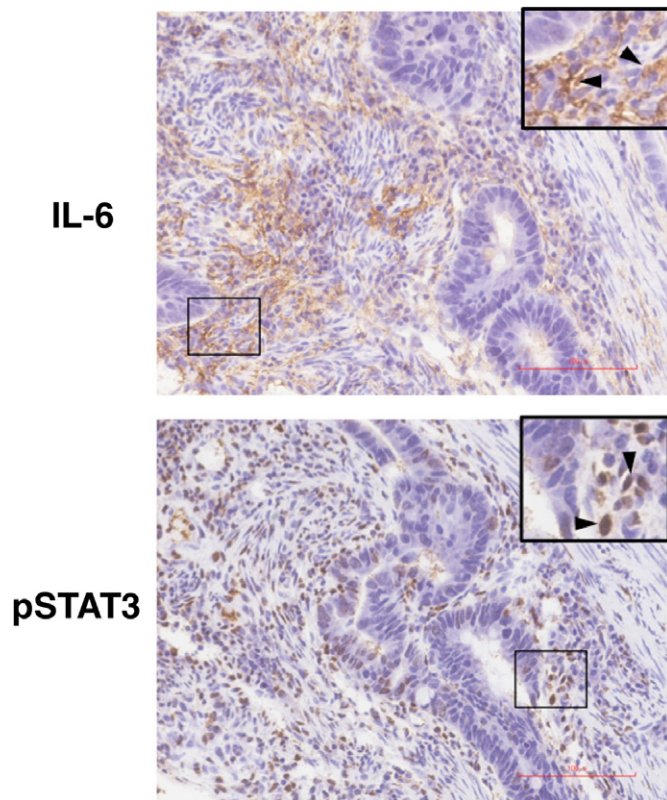
Fig. 1. Expression of interleukin (IL)-6 and phosphorylated STAT3 in tumor microenvironments of colorectal cancer patients. Cancer tissues obtained from colorectal surgical specimens were fixed in formalin and embedded in paraffin. Sections were treated with rabbit anti-human IL-6 or phosphorylated STAT3 antibodies overnight at 4°C. All sections were incubated with a peroxidase-labeled anti-mouse IgG polyclonal antibody or peroxidase-labeled goat anti-rabbit antibody as secondary antibodies. Protein expression was visualized using 3,3′-diaminobenzidine. Finally, sections were counterstained with Mayer's hematoxylin. Images are of representative cases of colorectal cancers. Representative images are shown. Bar = 200 μm.

Recently, we reported that IL-6 stimulation also reduces HLA-DR and CD86 expression levels in human DC induced from PBMC of healthy donors in a STAT3-dependent manner.[23] In this study, activation of arginase, lysosome proteases and cyclooxygenase (COX)-2 was involved in the downregulation of HLA-DR on CD11b+CD11c+ cells. We further confirmed that CD11b+CD11c+ cells in colorectal tumor tissues had reduced surface expression levels of HLA-DR and CD86 compared with those obtained from PBMC. *ARG1*, *CTSL* and *COX2* levels were increased in tumor-infiltrating CD11b+CD11c+ cells. In addition, tumor-infiltrating CD11b+CD11c+ cells impaired the T cell-stimulating ability compared with PBMC. 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.

A previous study has demonstrated that arginine is required for gene expression of MHC class II, and arginase activation induced by IL-6 causes dysfunction of DC in a tumor-bearing mouse model.[16] These data suggest that arginine is required for activation of the transcription of MHC class II gene in DC. Another previous report indicated that activation of the COX2/PEG2 cascade inhibits maturation of DC.[30] IL-6-conditioned immature DC as well as macrophages and MDSC are well known to show high levels of lysosome protease and arginase activities. Therefore, activation of COX2, as is the case in

arginase-1, may be involved in both degradation and transcriptional downregulation of HLA class II levels in DC.

In previous reports, CD14−HLA-DR^low/− cells were increased in the peripheral blood of advanced cancer patients, and these cells were defined as human MDSC.[31–33] A recent study has revealed that IL-11, a member of the IL-6 cytokine family, is produced in the tumor microenvironment of colorectal cancer patients and induces MDSC in a STAT3-dependent manner.[34] Taken together, we speculate that STAT3 activation in peripheral myeloid cells may systemically cause downregulation of the surface expression of HLA-DR. In addition to various immunosuppressive cytokines such as IL-10, IL-11 and TGF-β, IL-6 produced in the tumor microenvironment may be one of the main cytokines that control the expression of MHC/HLA class II in a STAT3-dependent manner. Therefore, IL-6/STAT3 signaling inhibits the maturation of tumor-infiltrating myeloid cells into antigen-presenting cells and attenuates subsequent T cell immune responses.

Suppressive Effects of Interleukin-6 on Dendritic Cell-mediated Th1 Cell Responses

The helper functions of antigen-specific Th1 cells, such as production of IL-2 and interferon (IFN)-γ, are essential to induce fully activated CTL in tumor-bearing hosts.[35,36] A previous report has demonstrated that a cancer antigen-derived peptide-containing helper epitope induces CTL efficiently *in vitro* according to the helper function of antigen-specific CD4+ T cells.[37] In fact, a cancer peptide vaccine containing a helper epitope induced CTL in an advanced cancer patient, and the patient experienced a clinical benefit.[38,39] Recent studies have revealed that IL-6 produced in tumor environments suppresses the differentiation of IFN-γ-producing Th1 cells and promotes subsequent tumor formation.[40–42] These results suggest that activation of Th1 cells is a crucial event for induction of anticancer immunity in cancer patients.

We demonstrated that the IL-6/STAT3 signaling pathway impairs the antigen-presenting function of human DC through downregulation of HLA class II expression, and attenuates Th1 immunity by decreasing IL-12 production from DC.[23] IL-6-conditioned monocyte-derived DC (MoDC) reduced the activation ability to induce cancer antigen-specific cytokine production by Th cells. IL-12 is an important cytokine for induction of Th1 immunity[43] because it activates STAT4 in CD4+ T cells, inducing subsequent IFN-γ secretion.[44] This study showed that IL-6/STAT3 signaling attenuates IL-12 production by MoDC and actually impairs IFN-γ secretion from CD4+ T cells *in vitro*. In previous reports, STAT3 was shown to regulate nuclear factor-κB recruitment to the IL-12p40 promoter in murine DC,[45] and activation of STAT3 inhibited *IL-12p35* gene expression in mice.[46] In fact, IL-12p35−/− mice easily develop tumors following carcinogen exposure compared with wild-type mice.[47] This report indicated that IL-12 is important for anticancer immunity through induction of Th1 immunity. Therefore, IL-6-mediated STAT3 activation in DC may attenuate anticancer immunity according to the Th1 immunity impaired by reduced IL-12 production (Fig. 2).

Blockade of the Interleukin-6 Signaling Cascade to Augment Dendritic Cell-mediated Antitumor Immunity

Dysfunction of the immune system in the tumor-bearing state is a critical issue for the development of effective cancer immunotherapies. A previous study has indicated that IL-6
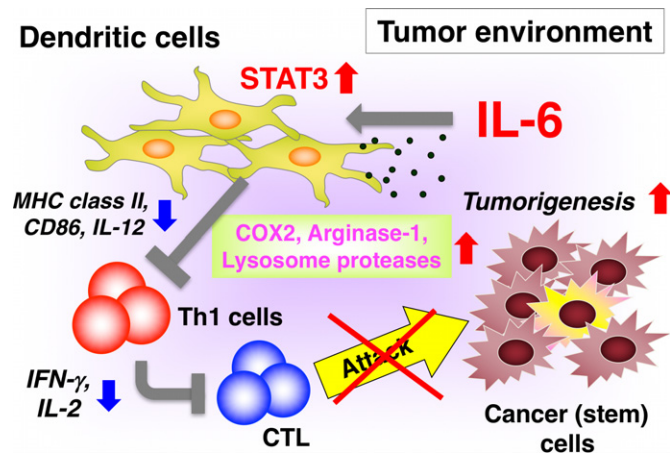
Fig. 2. Dysfunction of dendritic cells (DC)-mediated antitumor immunity induced by interleukin (IL)-6/STAT3 activation in tumor microenvironments. IL-6 produced in the tumor microenvironment suppresses major histocompatibility complex (MHC) class II and CD86 expression and IL-12 production of DC in a STAT3-dependent manner. Activations of COX2, arginase-1 and lysosome proteases are related to the downregulation of antigen-presenting ability of DC, which attenuates the subsequent induction of effector T cells, including Th1 cells and cytotoxic T lymphocyte (CTL). Dysfunction of antitumor immunity promotes *in vivo* tumorigenesis.

suppresses antigen-specific CD4+ T cell responses through downregulation of MHC class II on DC *in vivo*.[17,18] Based on these findings, we blocked IL-6 signaling by administration of a monoclonal antibody against IL-6 receptor (anti-IL-6R mAb) to enhance antitumor immunity, and confirmed its inhibitory effects on tumor growth.[19,20] In this study, we found that tumor-infiltrating CD11c+ DC enhanced the arginase-1 mRNA expression level and reduced surface expression of MHC class II in parallel with an increase in serum IL-6 levels at a late stage in tumor-bearing mice.

Furthermore, we demonstrated that N(ω)-hydroxy-L-arginine, an arginase-1 inhibitor, blocks the reduction in MHC class II levels on CD11c+ DC during the tumor-bearing state.[20] *In vivo* injection of the arginase inhibitor at a peritumor site significantly enhanced CD4+ T cell responses and inhibited tumor growth. Therefore, IL-6-mediated arginase activation and the subsequent reduction in MHC class II

expression may be involved in dysfunction of the DC-mediated immune system in tumor microenvironments. Accordingly, blockade of the IL-6-arginase cascade may represent a promising approach to overcome the dysfunction of antitumor immunity in tumor-bearing hosts (Fig. 2).

Other Aspects of Interleukin-6 for Anti-tumor Immunity and Tumorigenesis *in vivo*

Recent studies indicated that the generation of tumor antigen-specific effector T-helper cells was significantly attenuated, and impaired Th1 differentiation was restored by the temporal blockade of IL-6 activity at the T-cell priming phase. The researchers revealed that c-Maf activity was responsible for IL-6/sIL-6R-induced Th1 suppression and defective T-cell-mediated antitumor responses. These data suggest another mechanism for effects of IL-6 that will directly affect T cell function.[40–42]

Previous papers revealed that IL-6 promotes growth and epithelial–mesenchymal transition of CD133+ cells of non-small cell lung cancer,[48] tumorigenicity of oral cancer stem cells,[49] cell adhesion, cancer stem-like and metastatic spread of prostate tumors,[50] the growth and metastasis of breast cancer cells, renewal of breast cancer stem cells (BCSC), and drug resistance of BCSC.[51] Furthermore, it was reported that CD133+ cells produced IL-6,[48] suggesting that cancer stem cells may potentially reduce anti-tumor immunity *in vivo*. These findings indicate that IL-6 appears to play a critical role in tumorigenesis of cancer stem cells, suggesting that blockade of IL-6 signaling will be a promising target for the treatment and prevention of recalcitrant tumors.

Future Perspective

Recent clinical trials using a chimeric anti-IL-6 mAb, siltuximab, have been conducted for patients with metastatic renal carcinoma,[52,53] prostate cancer,[54,55] advanced solid tumors [56] and multiple myeloma.[57–59] These treatments were well tolerated by the cancer patients. Serum C-reactive protein was decreased and anemia caused by chronic inflammation was improved by administration of the anti-IL-6 mAb. These data suggest that blockade of IL-6 signaling might control the status of chronic inflammation in patients with advanced cancers. Recently, a phase I trial combining therapy using an anti-IL-
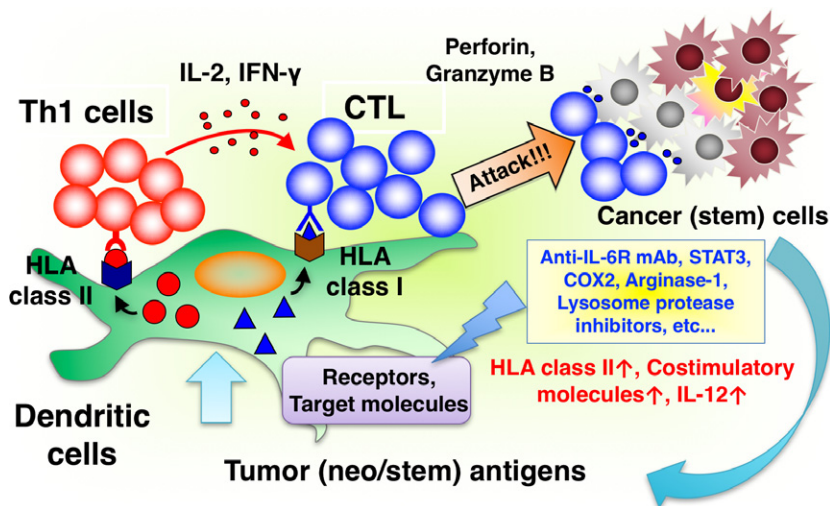

Fig. 3. Blockade of interleukin (IL)-6/STAT3 signaling is a promising strategy for enhancing dendritic cell (DC) functions in cancer immunotherapy. IL-6 produced in the tumor microenvironment suppresses antitumor immunity involving activation of Th1 cells and cytotoxic T lymphocyte (CTL) by causing dysfunction of DC, and promotes *in vivo* tumorigenesis by facilitating cancer cell growth in the niche. Therefore, blockade of IL-6/STAT3 signaling and the target molecules in DC by an antagonistic monoclonal antibody and inhibitors may be a promising strategy for the development of effective cancer immunotherapies in the near future.

6R mAb, tocilizumab, with carboplatin/doxorubicin and IFN-α2b has been conducted for patients with recurrent epithelial ovarian cancer.[60] This study reported that immune cells in the IL-6R mAb-treated patients had reduced levels of phosphorylated STAT3 and myeloid cells produced more IL-12, while T cells were more activated and secreted higher amounts of effector cytokines including IFN-γ. Patients receiving the highest dose of tocilizumab showed increased levels of serum soluble IL-6R, which was potentially associated with a survival benefit. Based on this evidence, we speculate that IL-6 may be a promising target for cancer immunotherapy through improving the antigen-presenting function of DC and facilitating maturation of antigen-presenting cells into tumor-infiltrating effector T cells against tumor-related antigens including neo/stem antigens from killed cancer cells (Fig. 3).

It has been reported that the presence of intratumoral T cells, including Th1, Th2 and Th17 cells, Tregs, and CTL, is a good prognostic factor in colorectal cancers.[61–64] We found that HLA class II expression levels of tumor-infiltrating immune cells are closely related to the invasion of CD4$^+$ T and CD8$^+$ T cells at tumor sites.[23] Therefore, blockade of IL-6/STAT3 signaling to enhance HLA expression on DC combined with other cancer treatments, such as cancer peptide vaccines, immunological adjuvants, immune checkpoint therapies and anticancer drugs, may show more clinical efficacy by restoring the T cell stimulatory ability of antigen-presenting cells in cancer patients.

Previous studies demonstrated that PD-L1 expression levels on cancer cells were augmented by stimulation with IFN-γ, which was generally produced by anti-tumor effector lymphocytes based on type-1 immunity in a STAT-1 dependent manner.[65–67] PD-L1-expressed cancer cells ordinarily reduce antitumor responses by PD-1-expressing effector cytotoxic T cells. IL-6-deficient condition increased Th1 and Tc1 immunity that may cause augmentation of PD-L1 expression levels on cancer cells and antigen presenting cells, including DC *in vivo*. Therefore, we speculate that blockade of IL-6/IL-6R signaling will facilitate immune-check point therapy using anti-PD-1/PD-L1.

TLR ligands such as poly I:C are promising tools to induce antitumor immunity by increasing IL-12 production and maturation of DC *in vivo*.[68,69] However, it has been reported that the TLR3/TRIF/MyD88-NF-kb signaling pathway in DC generally induces IL-6 as well as IL-12.[70] Therefore, we speculate that the IL-6-deficient condition promotes the antitumor effect of immunoadjuvant therapy using poly I:C.

Based on the present data and previous reports, we speculate that blockade of the IL-6 signaling pathway may promote introduction of antitumor immunity into the tumor-bearing host. Such an approach may be a promising strategy for the development of more effective immune checkpoint blockade therapies using anti-PD-1/PD-L1 mAbs and immunological adjuvants such as poly I:C for cancer patients.

The present review proposes that maintenance of the antigen-presenting ability of DC is one of the critical issues for the induction and activation of cancer antigen-specific T cells in the tumor microenvironment. Therefore, a therapeutic strategy to improve the function of DC by inhibition of the IL-6/STAT3 signaling cascade may be effective in increasing good responses to cancer immunotherapies.

Disclosure Statement

The authors have no conflicts of interest.

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