Tumor-Driven Evolution of Immunosuppressive Networks during Malignant Progression

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Abstract
Tumors evolve mechanisms to escape immune control by a process called immune editing, which provides a selective pressure in the tumor microenvironment that could lead to malignant progression. A variety of tumor-derived factors contribute to the emergence of complex local and regional immunosuppressive networks, including vascular endothelial growth factor, interleukin-10, transforming growth factor-β, prostaglandin E2, and soluble phosphatidylserine, soluble Fas, soluble Fas ligand, and soluble MHC class I–related chain A proteins. Although deposited at the primary tumor site, these secreted factors could extend immunosuppressive effects into the local lymph nodes and the spleen, promoting invasion and metastasis. Vascular endothelial growth factors play a key role in recruiting immature myeloid cells from the bone marrow to enrich the microenvironment as tumor-associated immature dendritic cells and tumor-associated macrophages. The understanding of the immunosuppressive networks that evolve is incomplete, but several features are emerging. Accumulation of tumor-associated immature dendritic cells may cause roving dendritic cells and T cells to become suppressed by the activation of indoleamine 2,3-dioxygenase and arginase I by tumor-derived growth factors. Soluble phosphatidylserines support tumor-associated macrophages by stimulating the release of anti-inflammatory mediators that block antitumor immune responses. Soluble Fas, soluble Fasl, and soluble MHC class I–related chain A proteins may help tumor cells escape cytolysis by cytotoxic T cells and natural killer cells, possibly by counterattacking immune cells and causing their death. In summary, tumor-derived factors drive the evolution of an immunosuppressive network which ultimately extends immune evasion from the primary tumor site to peripheral sites in patients with cancer. (Cancer Res 2006; 66(11): 5527-36)

Introduction
The host immune system is regulated by innate and adaptive immune responses to protect it from the attack of foreign entities, such as bacteria and viruses. Cancer cells can grow by escaping from the attack of immune cells, thus, disrupting the host immune system, which is progressively suppressed as a result of tumor progression and metastasis (1, 2). The molecular mechanisms by which cancer cells evade the host immune system have been investigated in mouse models and clinical samples. Several important mechanisms have been documented, such as those involving loss of tumor antigen (3), alteration of HLA class I antigen (4), defective death receptor signaling (5), lack of costimulation (6), immunosuppressive cytokines (7), and immunosuppressive T cells (8). The immunosuppressive cytokines, such as interleukin (IL)-10 and transforming growth factor-β (TGF-β), are secreted from tumor cells and inhibit the maturation of dendritic cells (DC) and T cell function through regulatory T cells (Treg; ref. 9). In addition to these tumor-derived cytokines, the small molecule, prostaglandin E2 (PGE2), inhibits T cell function as well as IL-10 and TGF-β (10).

Another important tumor-derived soluble factor (TDSF) is the vascular endothelial growth factor (VEGF), which acts as a chemoattractant for the production of immature myeloid cells (IMC) from the bone marrow, which are then recruited to the primary tumor site through the peripheral circulation in a mouse model (11). The IMCs produce immature dendritic cells (iDC) and macrophages, which are modulated by TDSFs in the tumor microenvironment to produce tumor-associated iDCs (TiDCs) and tumor-associated macrophages (TAM). The activities of indoleamine 2,3-dioxygenase and arginase I in TiDCs and TAMs are increased by TDSFs, such as IL-10 and TGF-β, to produce reactive oxygen species (ROS; refs. 12, 13); thereby, maturation of DCs and T cell proliferation are inhibited, which leads to apoptosis. The accumulation of TiDCs in the primary tumor site could be extended to the secondary lymphoid organs, such as regional lymph nodes and spleen—and the cells might possibly recirculate to the peripheral vessels—thereby providing cancer cells with an immunosuppressive network that extends the immune evasion and promotes tumor progression and metastasis (14).

Other TDSFs, such as soluble Fas ligand (sFasL) and soluble MHC class I–related chain A gene (sMICA) products, also play important roles in immune evasion, which inhibit Fas- and NKG2D-mediated killing of immune cells (15, 16). Another TDSF, soluble phosphatidylserine (sPS), acts as an inducer of an anti-inflammatory response to TAMs, resulting in the release of anti-inflammatory mediators, such as IL-10, TGF-β, and PGE2, that inhibit an immune response to DCs and T cells (17). The altered tumor surface antigen, such as Fasl, also causes immune evasion by counterattacking immune cells, leading to cell death (18). In addition, the soluble forms of Fasl and MICA, sFasl and sMICA, are able to inhibit Fas and the NKG2D-mediated death of immune cells (19, 20). Thus, it is likely that TDSFs play pivotal roles in constituting immunosuppressive networks that aid tumor progression and metastasis. Indeed, the immunosuppressive networks derived from these TDSFs can be a critical factor in causing the unsatisfactory clinical response that is usually seen in the immunotherapy of advanced cancer, and they remain an important obstacle to be overcome in the interaction between tumors and the immune system in the tumor microenvironment (1, 21). This article...
discusses the role of TDSFs in establishing the immunosuppressive networks that extend from the primary tumor site to the peripheral vessels, and how the immunosuppressive networks are critical obstacles to immunotherapy in patients with cancer. A better and more comprehensive understanding of the evolution of the immunosuppressive networks derived from tumor cells may provide new insights that may help break the immune suppression by cancer cells.

Tumor-Derived Factors Sculpt Inflammatory Processes to Promote Immune Evasion

Since the connection between inflammation caused by leukocytes in cancer tissue and progression of cancer was noted by Virchow in 1863 (22), studies of the inflammatory microenvironment of cancer tissues have helped to reveal the links between cancer and inflammation (23). Leukocytes infiltrate cancer tissues, particularly in the hypoxic tumor lesion, and the monocyte-macrophage lineage is a major component of the leukocyte infiltrate of tumors. Some cancers, such as liver, gastrointestinal, and gynecologic malignancies, in which the inflammatory process is a cofactor in carcinogenesis, are attributable to infections in these organs (24). Inflammatory mediators, including reactive oxygen and nitrogen intermediates, prostaglandins, and inflammatory cytokines, contribute to tumor development by inducing protumor mutations, resistance to apoptosis, and environmental changes, such as stimulation of angiogenesis, that confer a survival advantage to a susceptible cell.

Despite the fact that the progression and metastasis of cancer cells are mediated by proinflammatory responses in the tumor microenvironment, the immune response is regulated in anti-inflammatory situations by TDSFs such as sPS. Phosphatidylserine, an ATP-dependent aminophospholipid, triggers the phagocytosis of apoptotic cells by phagocytes such as macrophages and DCs. The clearance of apoptotic cells is mainly mediated by macrophages influenced by the interaction between phosphatidylserine and phosphatidylinositol receptor. Phagocytosis of apoptotic cells by macrophages occurs without the induction of inflammatory reactions (25, 26). The presence of apoptotic cells during macrophage activation has been shown to increase the secretion of anti-inflammatory mediators, such as IL-10, TGF-β, and PGE₂, and to decrease the secretion of proinflammatory cytokines such as IL-12 (27). Although phagocytosis of both apoptotic and necrotic cells is mediated by the phosphatidylserine/phosphatidylinositol receptor interaction, the reduced cytotoxicity of TAMs after ingestion of apoptotic cells may be explained by the anti-inflammatory response to cancer cells that was caused by several negative mediators. The sequential immune responses by TAMs and DCs can be abrogated by sPS derived from cancer cells. In the sequential interactions, the tumor-derived sPS reacts with phosphatidylserine receptor in TAMs and DCs to inhibit the innate immune response. The enforced anti-inflammatory response by sPS—not only by interaction with the phosphatidylserine receptor on TAMs, but also with the phosphatidylserine receptor on DCs to inhibit their maturation—may cause immune escape and tumor proliferation. Furthermore, the tumor-derived sPS bind to their own phosphatidylserine receptor, resulting in the production of IL-10, TGF-β, and PGE₂ to facilitate tumor proliferation.

During the anti-inflammatory response, the TGF-β derived from TAMs prevents the maturation of antigen-presenting DCs, thereby inhibiting the activation of natural killer (NK) cells and CTLs (28). In addition, TGF-β promotes the proliferation of stromal cells, such as fibroblasts and macrophages, and induces them to secrete angiogenic and cell survival factors that stimulate tumor proliferation, including cyclooxygenase-2 and VEGF through nuclear factor κB (29). These stromal cells also produce Th2 cytokines, such as IL-4 and IL-10, as well as PGE₂ as a result of TGF-β secretion. The increased PGE₂ concentration decreases the expression of MHC class I and II antigens in DCs (30). Under tumor-specific conditions, such as hypoxia and glucose starvation, macrophages produce hydroxide as well as TGF-β, and subsequently the oxidized macrophages, TAMs, also produce Th2 cytokines such as IL-4 and IL-10, which might be involved in immune escape (31). The increased levels of the cytokines, IL-4, IL-10, PGE₂, and TGF-β enhance the expressions of c-FLIP, Bcl-xL, and Mcl-1, which are involved in the resistance to apoptosis (32–34). Thus, the production of IL-10, TGF-β, and PGE₂ from TAMs—mediated by sPS or hydroxide—plays a critical role in immune escape. Because immune cells are suppressed during the anti-inflammatory response, the defective removal of apoptotic cells promotes an inflammatory response to cancer cells. In conjunction with the secretion of the cytokines, IL-10 and TGF-β, in the tumor microenvironment, cancer cells promote their own growth by activating antiapoptotic pathways concomitant with tumor angiogenesis activated by matrix metalloproteinase 2 (MMP-2) and MMP-9 (35, 36).

Role of VEGF and iMCs in Immune Evasion

VEGF is an important growth factor for endothelial cells in tumor angiogenesis, which is activated by hypoxia-inducible factor-1 in the tumor microenvironment (37). In addition, VEGF plays a critical role as a chemoattractant in the induction of iMCs from bone marrow (38). The induced iMCs, such as iDC and macrophages, flow into peripheral vessels, and they are recruited to primary tumor sites. These cells are either committed to apoptosis or modulated by TDSFs to become resistant to apoptosis in the tumor site with decreased Fas and increased Bcl-2 expression, which are attributed to TiDCs and TAMs (39). These tumor-associated iMCs are able to distribute to secondary lymphoid organs such as regional lymph nodes (e.g., sentinel lymph nodes) and spleen, where they are recirculated to peripheral vessels. In fact, the expansion of iMCs with immunosuppressive cell characteristics has been reported in patients with head and neck cancer (40), gastric cancer (41), and colon cancer (42). In the mouse tumor model, the iMCs are characterized by the expression of Gr-1 and CD11b markers, including iDCs, macrophages, and granulocytes. These cells suppress antigen-specific CD8+ T cells via direct cell-cell contact, mediated by ROS (43). Accumulation of H₂O₂ in these cells—in which arginase (Arg) activity plays an important role—was a major contributor to this increased pool of ROS. The iMCs could be induced by several TDSFs, such as IL-3, SDF-1, and VEGF from bone marrow, and then recruited to tumor sites through the peripheral circulation (44, 45). In particular, the TiDCs are resistant to apoptosis and show increased arginase I activity. Arginase I is an enzyme that converts the amino acid l-arginine to ornithine and urea, which inhibits T cell proliferation and functions in coordination with TGF-β. Furthermore, l-arginine is metabolized to nitric oxide and hydroxide by inducible nitric oxide synthase; the metabolites inhibit Th1 cytokine production and T cell function, and decrease expression of the T cell receptor-ζ (TCR-ζ) that leads to T cell apoptosis (46). Thus, VEGF-induced
iMCs play a critical role in inhibiting innate and adaptive immune responses in cancer cells. The induction of iMCs by tumor-derived chemoattractants is constitutive, whereas the induction of iMCs by bacterial infection and potent immunogens is transient and declined after several hours (47). In this regard, VEGF inhibited the differentiation of thymus precursors, resulting in thymus atrophy (48). It is likely that the constitutive induction of iMCs, their recruitment to the tumor site, and their functional modulation by TDSFs are critically involved in the evolution of the immunosuppressive network in patients with cancer.

NF-κB is a transcription factor that plays an important role in multiple cellular functions such as an immune response associated with an inflammatory response (49). Although NF-κB activation is required for the maturation of DCs and activation of T cells in an immune response, the activation of signal transducers and activator of transcription 3 in Gr-1 CD11b+ cells inhibits NF-κB activation as well as VEGF and PGE₂, whereby the maturation of DCs is blocked (50). In addition, the TAMs, freshly isolated from spleens of tumor-bearing or naïve mice, were able to inhibit the T cell–mediated immune response in vitro via induction of T cell apoptosis, which was responsible for signal transducer and activator of transcription 1 activation. Arginase and nitric oxide were both responsible for the apoptotic mechanism, and were present in TAMs (13). Of interest, coincubation of CD8+ T cells with Gr-1 CD11b+ cells immediately induced apoptosis of the T cells, whereas CD4+ cells were less sensitive to Gr-1 CD11b+-mediated apoptosis (51). One reason for the increased sensitivity of CD8+ T cells to Gr-1 CD11b+-mediated apoptosis may be explained by the observation that iMCs express MHC class I but very little or no MHC class II antigens. The population of iMCs is a heterogeneous mixture of precursors of different myeloid cells and early progenitors, therefore, whether the inhibitory effect of iMCs is limited to CD8+ T cells or is extended to CD4+ T cells may depend on the expression of MHC antigens on the iMC surface. In fact, the increased sensitivity of CD8+ T cells to apoptosis—which is due to the elevated Bax/Bcl-2 ratio on the T cells, Fas/Fas-ligand interactions, and high levels of FasL, expression on the tumor—have been observed in patients with head and neck cancer (52), breast cancer (53), and oral cancer (54). Furthermore, microvesicle-associated FasL-induced decreased expression of TCR-ζ, which is a substrate of caspase-3, leads to altered signal transduction in T cells and apoptosis (55). The altered T cell dysfunction was attributed to the induction of apoptosis due to ROS and hydroxide derived from l-arginine because T cells stimulated and cultured in the absence of l-arginine present a sustained down-regulation of TCR-ζ, exhibit a decreased rate of proliferation, and a significantly diminished production of IFN-γ (46). Although inhibition by iDCs of T cell function is mediated by cell-cell contact, iDCs produce anti-inflammatory cytokines, such as IL-10 and TGF-β, that lead to an increase in arginase I activity, resulting in the production of ROS, which is involved in the inhibition of T cell function (43). The functional roles of TDSFs and iMCs are summarized in Fig. 1.

**Constitution of an Immunosuppressive Network Initiated from the Primary Tumor Site**

The accumulation of iDCs is found not only at the primary tumor site but could also be extended to the secondary lymphoid organs and peripheral vessels, which facilitates constitution of the immunosuppressive network that supports tumor growth in patients with cancer. In fact, increased numbers of iDCs have been shown in lymph nodes and peripheral blood in patients with head and neck, breast, and non–small cell lung cancers according to their tumor progression, and these iDCs are able to inhibit DC maturation and T cell function (56, 57). The TDSF-induced new iDCs from bone marrow are recruited to the primary site and are educated by biochemical modulation to show, for example, resistance to apoptosis and increased activity of arginase I. The TiDCs may be different from tumor-associated DCs because the latter are committed to apoptosis. However, some TiDCs are converted to mature tumor-associated DCs, and some of them may be able to activate T cells as antigen-presenting cells. In contrast, TiDCs are able to exert immunosuppressive activity that affects not only the primary tumor site but also secondary lymphoid organs, including regional lymph nodes and spleen, and extends to peripheral vessels. This immunosuppressive network is associated with tumor progression and affects the poor prognosis for patients with advanced cancer. The evolution of immunosuppressive activity in cancer patients is also revealed in the sentinel lymph nodes of patients with melanoma (58) and breast cancers (59, 60). A high degree of down-regulation of TCR-ζ is observed in the sentinel lymph nodes of patients with breast cancer (61). Furthermore, in our preliminary study we observed that expression of the costimulatory molecules, such as CD80 and CD86 in DCs, was down-regulated in the sentinel lymph nodes in breast cancer patients, and the expression of CD83 was also decreased, indicating that the increased iDCs in the sentinel lymph nodes might play a critical role in an evolving immune evasion. In addition, given that increased levels of IL-10 and decreased levels of IL-12 were observed in the sentinel lymph nodes, the Th2-dominant situation also contributes to immune evasion in the sentinel lymph nodes.4

The spleen is a secondary lymphoid organ that can be involved in an antitumor immune response. In the second half of the 1970s, the immune suppressor cells, including DCs and T cells, were first described in the mouse tumor model (62, 63). The number of these suppressor cells in the spleen increases together with tumor growth, and they are now identified as plasmacytoid DCs (pDC; ref. 64) and CD4+ CD25+ Tregs (65). Some TiDCs can be converted to pDCs, the pDCs are being recruited to tumor sites, mediated by CXCR4/CXCL12 (SDF-1) interaction between pDCs and tumor cells. Tumor-derived CXCL12 mediates the trafficking of pDCs that express CXCR4 to the tumor (45). pDCs originate in iMCs in the bone marrow; iMCs are induced by TDSFs, such as VEGF, that are recruited to tumor sites through interaction with chemokines and chemokine receptors, such as the CXCR4/CXCL12 interaction. CXCL12 protects tumor pDCs (TpDC) from apoptosis, these cells can be phenotypically and functionally modulated in the tumor microenvironment (45). Functional pDCs are found in the local tumor microenvironment of patients with ovarian cancer (45), melanoma (66), and head and neck squamous cell carcinoma (67). TpDCs induce significant production of IL-10 by T cells that suppress myeloid DC (mDC)-induced tumor antigen–specific T cell effector functions. Despite the fact that activated peripheral blood pDCs stimulate tumor-specific T cell and IFN-γ production, TpDCs show reduced expression of toll-like receptor 9 (TLR9) for inducing IFN-α. CXCR4-CXCL12 signals are implicated in the trafficking of pDCs into tumors, and they are further involved in tumor vascularization and metastasis by evoking immune evasion through functional modulation of the pDCs.

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4 Unpublished results.
CD4+ CD25+ Tregs play a crucial role in regulating the immune response and in maintaining immune homeostasis (68). Naturally occurring Tregs represent a subset of Tregs that suppress immune responses through a cell-cell contact mechanism without specific antigen stimulation (68). In comparison, antigen-induced Tregs mediate immune suppression either through cell-cell contact or through secretion of soluble factors such as IL-10 and TGF-β (69). The function of Tregs, which are involved in establishing immunologic tolerance in cancer immunosuppression, has been reported to be the inhibition of CD8+ and CD4+ T cells. Several studies have reported increased frequencies of CD4+ CD25+ Tregs in blood, malignant effusions, draining lymph nodes, and tumor tissues, thus, associating an impaired immune response to cancer with a high frequency and/or hyperactivity of Tregs (8, 70). The immunosuppressive cytokines, such as IL-10 and TGF-β, derived from tumor cells, induce Tregs (71), which activate the activity of indoleamine 2,3-dioxygenase for the inhibition of T cell function. Furthermore, immature DCs tend to induce immune tolerance rather than immunity by either deleting reactive T cells or inducing Tregs (72). The functional and molecular characterization of Tregs has been made possible by the recent association of cell markers with immunoregulatory activity—such as the tumor necrosis factor (TNF) family molecule GITR (73), CTL antigen-4 (74), and Foxp3 (71)—which affect both antitumor immunity and autoimmunity.

Role of Altered Tumor Surface Antigens and Their Soluble Factors in Immune Evasion

The cytolytic function of CTLs and NK cells in killing tumor cells is mediated by either the death receptor–dependent pathway or perforin-induced granzyme B, a serine protease, which leads to apoptosis. The Fas-dependent cell death pathway is regulated by the Fas receptor on tumor cells and its ligand FasL on CTLs. The soluble form of Fas, sFas, which is derived from the cleavage of Fas by MMPs in tumor cells, is increased according to tumor progression in patients with breast cancer (75) and other malignancies (76, 77). Tumor-derived sFas can interact with asL on CTLs, whereby Fas-mediated apoptosis is inhibited. In addition, the altered expression of FasL on tumor cells is able to counterattack CTLs, leading to their apoptosis (78). This mechanism may also activate FasL-mediated activation-induced cell death for CTLs (79). The soluble form of FasL, sFasL, which is
derived from cleavage of FasL by MMPs, can also counterattack CTLs but to a lesser extent than FasL (80). In fact, tumor cells of various cellular origins were sensitized to Fas-mediated cytotoxicity by MMP inhibitors (81). sFasL is also able to inhibit the interaction of Fas/Fasl between tumor cells and CTLs in Fas-mediated apoptosis. Furthermore, internalization of sFasL into Fas in tumor cells down-regulates Fas expression, which contributes to the development of resistance to Fas-mediated apoptosis. Thus, the altered expressions of FasL and the soluble factors, sFas and sFasl, contribute to immune evasion by allowing tumor cells to escape from immune attack by CTLs.

The other effectors, NK cells, play an important role in antitumor immune responses via NKG2D-mediated apoptosis (82). This pathway is regulated by the interaction between the NKG2D receptor on NK and CD8+ T cells, and its ligand, MICA on tumor cells. The soluble form of MICA (sMICA), derived from tumor cells, could inhibit NKG2D-mediated apoptosis by interfering with the NKG2D/MICA interaction between tumor and NK cells (83). Tumor-derived sMICA internalizes with the NKG2D receptor on NK cells, resulting in the down-regulation of the expression of NKG2D, thereby decreasing NK-mediated killing of MICA+ tumor cells (84). Thus, it is likely that not only the soluble death receptors, such as sFas derived from tumor cells, but also the altered expression of death receptor ligands, such as FasL and MICA and their soluble forms, play a role in immune evasion by tumor cells (Fig. 2).

**Clinical Implications of Disruption of the Immunosuppressive Network**

Given that the evolution of the immunosuppressive network from the primary tumor site plays a critical role in maintaining immunosuppression in cancer patients—mediated by TDSFs and altered tumor surface antigens according to tumor progression—the simplest and best way to eliminate the immunosuppressive network is by removal of the primary tumor and eradication of tumor cells from the cancer patient. However, despite complete surgical resection of a primary tumor with regional lymph node dissection, residual tumor cells may still exist, and these tumor cells would be able to grow and reconstitute the immunosuppressive network during tumor progression. It is conceivable, though, that residual tumor cells after surgical resection may be completely removed by adjuvant therapy such as chemotherapy and immunotherapy. Complete eradication of residual tumor cells by chemotherapy can increase the cure rate of patients with cancer, and activation of the antitumor immune response may provide an increase in cure rate either by complete eradication of tumor cells or prolongation of disease-free survival by controlling the number of residual tumor cells following surgical resection. Unfortunately, the potential contribution to survival benefit of effective immunotherapy as an adjuvant after surgical resection has not yet been established in solid tumors. Nevertheless, immune activation by massive tumor cell death after neoadjuvant chemotherapy may be effective in antagonizing the anti-inflammatory response in immune cells, which disrupts immune tolerance mediated by iMCS. Induction of massive cell death, including apoptotic and nonapoptotic cell death, activates the immune response, which is mediated by the engulfment of dead cells by DCs and increased release of tumor antigens or tumor-associated antigens to secondary lymphoid organs such as sentinel lymph nodes and spleen. Because the sentinel lymph nodes in breast cancer are involved in immune suppression, activation of T cells through cross-priming by mature DCs enables them to attack tumor cells in the primary and other tumor sites. Therefore, activation of the immune response in sentinel lymph nodes may result in not only a tumor response but also prolongation of survival after neoadjuvant chemotherapy. Similarly, the immunosuppressive myeloid cells accumulated in the spleen of patients with advanced gastric cancer may be modulated to provide immune activation after neoadjuvant chemotherapy. Thus, the potential immunostimulation after neoadjuvant chemotherapy for solid tumors, such as breast and gastrointestinal cancer, will be of great interest. The molecular mechanism of the immune activation and its clinical significance will also need to be clarified in future studies.

Over the past two decades, several modalities for immunotherapy, such as administration of cytokines, transfer of T cells and DCs, have been tried in various types of cancer patients, and a recent development of specific cancer vaccines as adoptive immunotherapy has emerged. Although a number of clinical studies have been conducted for various types of cancer, the clinical response rate was only ~2.6%, hence, a cancer vaccine is not yet a reality (21). The reason for the low response rate may be that the clinical trials were conducted on advanced cases and that most of them had failed previous chemotherapy. In these situations, the immune evasion that originated at the primary tumor site and extended via its suppressive network to secondary lymphoid organs and peripheral vessels could not be disrupted by vaccine treatment alone, even though a novel tumor antigen was used for immune activation. Current clinical trials have shifted to combination therapy with vaccine together with anticancer drugs or radiation. The rationale for using combination treatment is that massive tumor cell death may be expected, followed by immune activation with loading of tumor antigens and tumor-associated antigens. Because a key factor in an immune response is the release and loading of tumor antigens and tumor-associated antigens derived from massive tumor cell death and subsequent activation of DCs, it is likely that massive tumor cell death is critical for generating an effective immune response against the anti-inflammatory response to immune cells. In general, tumor antigens and tumor-associated antigens are down-regulated in stromal cells in the tumor microenvironment because the stromal cells, such as the tumor-associated fibroblasts surrounding tumor cells, increase interstitial fluid pressure (IFP), which inhibits the release of tumor cells and antigens to secondary lymphoid organs. Furthermore, the extracellular matrix binds tumor antigen (83), and fibroblasts and endothelial cells compete with DCs for the antigen (86), whereby many tumor antigens are down-regulated, thus allowing tumor progression (87). A decrease in IFP following massive tumor cell death facilitates the release and loading of tumor antigens, thereby activating immune cells (Fig. 3). Although apoptotic cells are engulfed by DCs, and the tumor-associated antigens are processed for MHC class I antigen cross-presentation to CD8+ T cells, causing their activation (88), necrotic cells are proinflammatory and may be more suitable than apoptotic cells for the activation of T cells (89). In addition, nonapoptotic cell death, autophagy, is attributed to the presentation of MHC class II antigen for the regulation of CD4+ T cells (90). It is unclear which type of cell death is most applicable for the activation of T cells and the generation of an antitumor immune response in a clinical situation.

Given that VEGF plays a dual role—by being involved in tumor angiogenesis and functioning as a chemoattractant inducing iMCS from bone marrow—the blockage of VEGF by anti-VEGF antibody
could modulate the immunosuppressive network that is initiated from the primary tumor site (91). Continuous recombinant VEGF infusion resulted in the dramatic inhibition of dendritic cell development, associated with an increase in the production of B cells and immature Gr-1(+) myeloid cells (11). Infusion of VEGF was associated with inhibition of the activity of the transcription factor NF-κB in bone marrow progenitor cells. VEGF itself affected polypotent stem cells, resulting in the observed abnormal hematopoiesis. In contrast, treatment with anti-VEGF antibody decreased the accumulation of iMCs from bone marrow and improved DC function in tumor-bearing hosts (92). However, the inhibition of VEGF is a partial effect because soluble VEGF cannot completely be blocked by anti-VEGF antibody. Nevertheless, the inhibition of VEGF as an inducer of iMCs may be valuable for recovering the immune response, particularly in combination with anticancer drugs. In addition, it should be noted that treatment with anti-VEGF antibody decreases IFP, which may enhance the immunomodulatory action of anti-VEGF antibody in combination treatment (93). Recent reports of clinical trials for colon cancer (94), non–small cell lung cancer (95), and breast cancer (96) revealed the substantial survival benefits of bevacizumab, a humanized anti-VEGF monoclonal antibody, in combination with standard chemotherapy. The reproducible survival benefit of bevacizumab for various types of solid tumors may be explained by the decrease in accumulated TiDCs and TAMs and their functional inhibition, which could facilitate the activation of DCs and T cells following tumor cell death by anticancer drugs.

Because the immune cells in tumor-bearing hosts are regulated as part of the anti-inflammatory response, a proinflammatory response to DCs is necessary for the activation of a tumor-specific immune response. One possible approach is the use of cytosine-phosphate-guanosine (CpG) oligodeoxyribonucleotides (ODN), among which, CpG-DNA was the first one shown to possess an immunostimulatory effect in cancer patients treated with a bacterial lysate (97). This effect is induced by unmethylated CpG dinucleotide, which is a specific base pair sequence that conferred immunostimulatory activity to bacterial DNA and synthetic ODNs containing these same sequences (98). The recognition of the specific CpG motif is mediated by TLR9 as part of an effector mechanism (99). CpG activates macrophages, DCs, B cells, and NK cells, causing increases in various factors such as IL-1, IL-6, IL-12, TNF-α, IFN-α, costimulatory molecules, MHC class II, and IFN-γ for innate immune responses (Fig. 4). CpG 7909, a class B type CpG ODN, which is characterized by a phosphorothioate backbone without poly-G tails, is being tried as an adjuvant in a phase II trial for lung cancer. A recent report indicated that patients with

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**Figure 2.** Possible roles of sFas, sFasL, and sMICA in promoting immune escape. Tumor-derived sFas interacts with FasL on CTL, resulting in the inhibition of Fas-mediated cell death. FasL and sFasL are able to counterattack CTLs, which leads to the apoptosis of CTLs, and sFasL competes with FasL of CTLs for binding to tumor cells. Tumor-derived sMICA internalizes with the NKG2D receptor on NK cells, which leads to its down-regulation and results in the inhibition of NKG2D-mediated cell death. sMICA competes with MICA for binding to NK cells. Summary: TDSFs, sFas, sFasL, and sMICA help tumor cells escape Fas and NKG2D-mediated cell death by competing with FasL of CTLs and MICA of tumor cells.
melanoma who received vaccinations of CpG 7909 mixed with a melanoma antigen A analogue peptide, and incomplete Freund’s adjuvant, exhibited rapid and strong antigen-specific T cell responses: the frequency of Melan-A-specific T cells increased to >3% of the circulating CD8+ T cells, which was one order of magnitude higher than the frequency seen in control patients treated similarly but without CpG (100). Furthermore, combination treatment with CpG ODNs and anticancer drugs or radiation enhanced the therapeutic effect in an orthotopic tumor model (101, 102). In addition, antisense Bcl-2 (AS Bcl-2, oblimersen sodium), containing two CpG motifs, induced an immunostimulatory response, as shown by splenomegaly and an increase in IL-12 in a mouse model, which may be shifted to a Th1 immune response (103). In fact, a recent study showed an increase in IgM level over time for responders among patients with relapsed multiple myeloma treated with AS Bcl-2 in combination with dexamethasone and thalidomide (104). Although potential survival benefits of AS Bcl-2 in combination with anticancer drugs have been suggested for metastatic melanoma and chronic lymphocytic leukemia, the clinical relevance still remains to be determined. Further clinical studies are being continued for solid tumors and other malignancies to clarify the clinical efficacy of AS Bcl-2 in cancer therapy.

Concluding Remarks
Cancer immune evasion initiated from the primary tumor site, which is mediated by TDSFs and altered tumor surface antigens, is able to extend to secondary lymphoid organs and peripheral vessels, resulting in the formation of an immunosuppressive network that inhibits an antitumor immune response. The increased number of iMCs recruited from the bone marrow, triggered by VEGF and other TDSFs, as well as their TiDCs and TAM, play a critical role in suppressing the immune response at not only the primary site but also at secondary lymphoid organs and peripheral vessels. Given that tumor cells are regulated by a proinflammatory mechanism that stimulates tumor growth, whereas immune cells are regulated by an anti-inflammatory mechanism that causes immune suppression, proinflammatory or anti-inflammatory treatments have been controversial and considered as a double-edged sword in cancer therapy. However, because a proinflammatory response is mediated by massive tumor cell death, and other immunostimulatory agents are crucial for provoking an antitumor immune response, a proinflammatory response does not necessarily promote tumor growth. In contrast, anti-inflammatory treatment with, for example, NF-κB inhibitor may affect the activation of immune cells. Selective targeted

Figure 3. Role of IFP in immune evasion. The bone marrow–derived myeloid cells, such as iDCs and TAMs, and non-bone marrow–derived cells, such as fibroblasts, endothelium, and extracellular matrix, increase the IFP. The increased IFP subsequently decreases the tumor antigen level because extracellular matrix binds to antigen, and endothelium/fibroblasts compete with DCs for antigen, which leads to immune ignorance. Massive tumor cell death induced by anticancer treatment decreases IFP, resulting in an increased release of tumor cells and tumor antigens to secondary lymphoid organs. Nevertheless, accumulation of iDCs in sentinel lymph nodes inhibits the maturation of DCs and a tumor-specific T cell response, which leads to immune tolerance. Summary: nontumorous cells increase IFP, which decreases tumor antigen level and causes immune ignorance. Massive tumor cell death decreases IFP and provokes a tumor-specific immune response.
therapy for effects downstream of NF-κB may be more effective than nonspecific inhibition of NF-κB. Thus, primary cancer therapy needs to be focused on inducing a proinflammatory response, which can be connected to the activation of an immune response to tumor antigens and tumor-associated antigens in cancer cells.

Many previous reports indicated that tumors infiltrated with lymphocytes or mature DCs had a good prognosis. Furthermore, recent reports on the use of DNA microarrays for gene expression analysis also suggested that an increase in expression of immune response–related genes is associated with a good prognosis (105, 106). Despite the fact that treatment with anticancer drugs and molecular targeting agents induces infiltration of T cells into the tumor, the molecular mechanism by which anticancer treatment activates an immune response against the tumor-induced immunosuppressive network in individual cancer patients still remains to be elucidated. Further studies will be required for the analysis of individual immune responses to tumor cells following anticancer treatment to disrupt and modulate the immunosuppressive network. Such studies may provide new insights and help to overcome a critical obstacle in cancer therapy.

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