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Tumor-Infiltrating Dendritic Cells in Cancer Pathogenesis

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Dendritic cells (DCs) play a pivotal role in the tumor microenvironment, which is known to affect disease progression in many human malignancies. Infiltration by mature, active DCs into the tumors conveys an increase in immune activation and recruitment of disease-fighting immune effector cells and pathways. DCs are the preferential target of infiltrating T cells. However, tumor cells have means of suppressing DC function or of altering the tumor microenvironment in such a way that immune-suppressive DCs are recruited. Advances in understanding these changes have led to promising developments in cancer-therapeutic strategies targeting tumor-infiltrating DCs to subdue their immune-suppressive functions and enhance their immune-stimulatory capacity. The Journal of Immunology, 2015, 194: 2985–2991.

In recent years, it has become increasingly appreciated that the immune system’s role in modulating malignancy is far more complex than anticipated. A number of studies found correlations between the presence of infiltrating immune cells in the tumor microenvironment (TME) and prognosis of many cancers, such as ovarian, renal cell, colorectal, and breast cancers (1). The immune component of the TME consists of predominantly CD4+ and CD8+ T cells, dendritic cells (DCs), macrophages, and regulatory T cells (Tregs) (2). In general, T cell infiltration portends a better outcome (1, 3–5). One key example, published by Zhang et al (1), found that tumor-infiltrating T cells were observed in 55% of tumors obtained from advanced ovarian cancer patients. The 5-y overall survival rate for patients whose tumors contained tumor-infiltrating T cells was 38% in comparison with a 4.5% rate of survival for those whose tumors did not. In contrast, many other studies over the past decade demonstrated that other subsets of adaptive immune cells are typically, but not always, associated with worse prognosis, seeming to promote tumorigenesis (6–9). For example, Tregs (CD4+/CD25+/FOXP3+) in ovarian cancer confer a significantly higher risk for death, even when controlled for stage and degree of surgical reduction of disease (8).

In addition to immune-suppressive T cells, tumors by themselves are adept at preventing the destructive capabilities of infiltrating antitumor immune effector cells. Tumors promote apoptosis and paralyze antitumor effector cells through the release of immune-suppressive factors like NO, IL-10, IL-6, arginase-I, vascular endothelial growth factor (VEGF), IDO, and TGF-β (10–14).

Also, suppressive cells of the innate arm of the immune system, such as inflammation-induced myeloid-derived suppressor cells and tumor-associated macrophages, are known to be correlated with poor outcome and rapid disease progression (15–22). Although the negative roles of these innate immune-suppressive cells in the TME are widely demonstrated, the role of others, such as DCs, has been subject to debate because of conflicting observations (23–26). DCs have an integral role in influencing the immune response and are the subset of cells in the TME to which antitumor T cells are attracted; however, they may alter their role from being immunostimulatory to immunosuppressive at different stages of cancer progression (27). The focus of this article is on tumor-infiltrating DCs (TIDCs). We discuss their interaction with the progression or suppression of malignancy and highlight the new directions for the therapeutic manipulation of such immunosuppressive DCs to tip the balance in favor of antitumor immunity.

Dendritic cells

Described in the early nineteenth century by Paul Langerhans and termed “dendritic cells” in 1973 by Ralph M. Steinman and Zanvil A. Cohn, DCs are key decision makers, determining whether the adaptive arm of the immune system should or should not be activated. Crucial as professional APCs, they present Ags and provide a multitude of other necessary signals (costimulatory molecules and cytokines) for T cell activation and differentiation, thereby shaping the immune response. DCs also interact with other immune cells, including NK cells and B cells (28, 29). Many subsets of DCs with unique and specific functions, morphology, and localization have been described (30). These include Langerhans cells, monocyte-derived DCs (CD14+ DCs), myeloid DCs, and plasmacytoid DCs (pDCs). Furthermore, each of these
has different maturation states that add to the complexity. Identification of DCs, because of their heterogeneity, can be challenging and variable between studies, with some using single surface cell markers and others using multiple surface cell markers. At least three subsets of splenic murine DCs have been described: CD11chighCD8α+CD11b+DEC205+ lymphoid DCs, CD11chighCD8α–CD11b+DEC205+ myeloid DCs, and CD11cintermediateCD8α–/–CD11b–B220+Gr-1+ pDCs (31). Generally, myeloid DCs are thought to exhibit stimulatory effects, whereas lymphoid DCs or pDCs are involved with regulation or tolerogenesis. However, there are reports demonstrating immunosuppressive activities on the part of myeloid DCs and Ag presentation by pDCs (32). DC lineage is not as well characterized in humans; they are thought to arise from a myeloid progenitor, shared with macrophages, called a macrophage–DC progenitor, which, in turn, gives rise to common DC progenitors (CDPs). CDPs then give rise to pDCs and pre-DCs that are progenitors of two myeloid subsets. Human pDCs express surface markers CD123, CD303 or BDCA-2, and CD304 or BCDA-4. Human myeloid DCs are defined largely by CD11c/BDCA-1+ or CD141/BDCA-3+ expression. There are many conserved molecular pathways when comparing murine and human DCs, suggesting preservation of function (33).

DCs tend to congregate in blood and lymphoid tissues, although they are found widely in the body, as Langerhans cells in the skin and as intestinal DCs in the gastrointestinal tract. The characteristics of human TIDCs have perhaps best been studied in cutaneous melanoma. Noted though, is the difficulty of clearly characterizing human TIDCs because of significant patient/population heterogeneity, relatively low frequency of TIDCs, and differences in the use of markers to assess TIDC populations. In melanoma, TIDC frequency tends to be higher in the peritumoral area, and those cells exhibit a more mature phenotype, whereas more immature TIDCs are found within the tumors. However, the relationship between TIDC location and clinical outcome is not well understood, because it depends on TIDC type and communication with other cells (34). In general, mature DCs have been considered immunostimulatory, whereas immature ones have been considered suppressive and tolerogenic. However, based on their attributes, such as activation status, maturity, and polarization in TME, DCs’ functional plasticity is considered complicated (35–39). This makes it difficult to make any generalized inference about the roles of DCs in the TME.

**TIDCs**

**TIDCs have an immature and/or paralyzed phenotype.** TIDCs have been found in the TME in many cancer types, such as breast, colorectal, lung, renal, head and neck, bladder, gastric, and ovarian (40). Not surprisingly, because of the complexity of phenotype, as well as the methods of identification, DC infiltration into tumors was reported to have an association with both good and poor prognosis in different tumor types. For example, in one study, patients with colorectal carcinoma and high numbers of TIDCs exhibited shorter disease-free and overall survival than did those with low numbers (41). Conversely, TIDC presence was correlated with regression of melanoma (32). Prognostic impact may be more related to a shift in TIDC phenotype or the relative proportions of subtypes within the tumor infiltrate, rather than simply the degree of TIDC infiltration into the tumor (42). TIDCs tend to exhibit a phenotype of low costimulatory molecule expression (26, 37), blunted Ag cross-presentation (26), and high expression of regulatory molecules and receptors (37, 43) and are usually associated with immunosuppression. Animal modeling studies showed that the type, phenotype, and amount of TIDCs are dynamic over time and may influence disease progression significantly at different stages of tumor growth. For example, Krempski et al. (37) showed in an ID8 mouse model of ovarian cancer that, as the cancer progresses from light to heavy tumor burden, the numbers of TIDCs increase. Nearly all of the DCs progressively upregulated immune-suppressive molecules; this change is associated with a concomitant loss of T cell infiltration (37). Scarlett et al. (38) made similar observations in a spontaneous murine model of ovarian cancer. In that model, tumor progression was associated with increasingly dense immune infiltrates, which included DCs, macrophages, myeloid-derived suppressor cells, and T cells. A turning point was observed, at which aggressive growth correlated with a switch from immune-stimulatory DCs to immune-suppressive DCs. Depletion of DCs in that model early in the course of disease led to increased tumor expansion and aggression, whereas depletion later led to abrogation of tumor growth, suggesting that tumors eventually develop strategies to prevent DCs from sensing endogenous danger signals and responding with immune stimulation. Although it is not possible to determine whether this is relevant to human cancer, there are some indications in the literature. For example, Kocián et al. (44) found, in a study of colorectal cancer, that relapsed patients were more likely to have had fewer TIDCs in their primary tumors. Importantly, there was a distinct difference in the proportion of mature and immature (i.e., tolerogenic) TIDCs noted, with patients experiencing disease recurrence having higher densities of immature TIDCs and lower densities of mature TIDCs in their primary lesions. A study of non–small cell lung cancer TIDCs demonstrated that there are three DC subsets: those with high, intermediate, and no CD11c expression. In particular, the intermediate CD11c–expressing group appeared to have low levels of costimulatory molecules and high levels of the immune-inhibitory molecule programmed death ligand 1 (PD-L1) (25). TIDCs as a group exhibited poor response to TLR stimulation in terms of Ag-presentation capability. In breast cancer, pDCs within the primary tumor correlated with worse prognosis. When isolated from breast tumor biopsies from patients with high mitotic index and triple-negative breast tumors (aggressive breast tumors), pDCs produced low amounts of IFN-α in comparison with pDCs isolated from patients’ blood. These cells also sustained FOXP3+ Treg expansion, potentially contributing to immune tolerance and worsened clinical outcomes (45). Although it is not yet clear what specifically induces these shifts in DC subset distribution and/or phenotypic shift, release of cytokines or other factors into the TME are likely involved.

**Local induction of paralysis of TIDCs.** Tumor cells and the TME release factors that inhibit or reverse DC maturation and normal function. For example, Michielsen et al. (46) showed that conditioned media obtained from culturing human colorectal tumor explant tissue were high in VEGF and the chemokines CCL2, CXCL1, and CXCL5. Pretreatment of DCs in vitro with this media resulted in inhibition of maturation. A dynamic network, consisting of cell surface
molecules and soluble factors, regulates TIDC-mediated tumor-immune interactions in the TME (Fig. 1). Our group observed that one way in which tumors paralyze DCs is through the induction of PD-1 expression (37). PD-1 and its ligands PD-L1 and PD-L2 constitute an important immune-regulatory pathway that suppresses or impairs effective T, B, and myeloid cell responses in the initiation (priming) and effector phases of the immune response. Our data show that PD-1 expression on murine DCs is minimal in the early stages of tumor growth; however, as the disease progresses, nearly all TIDCs eventually have high levels of PD-1 (37, 47). Blockade of PD-1 on these TIDCs in vitro enhanced the production of immune-stimulatory cytokines, increased NF-κB activation in DCs, improved costimulatory molecule expression, and improved the ability of these DCs to activate T cells (37, 47). Thus, the paralyzed phenotype of TIDCs is likely reversible, at least pharmacologically. Another mechanism by which TIDCs are locked into an immune-suppressive or nonimmunogenic phenotype is through the upregulation of T cell Ig and mucin domain 3 (TIM-3) at the cell surface. TIM-3 has been largely studied as a suppressive marker of Th1 type T cells, and blockade of TIM-3 exacerbates various T cell–mediated autoimmune diseases (48). More recently, its expression and role in modulating innate immune activation have become clearer (49). Chiba et al. (50) found that both murine and human tumors induce upregulation of TIM-3 on DCs through multiple factors found in the TME, such as IL-10, TGF-β1, VEGF-A, IDO, and arginase. More importantly, they found that TIM-3 interacted with HMGB1 and prevented it from sensing tumor-derived nucleic acid danger signals in the TME. In addition to directly suppressing danger signal sensing, TIM-3 ligation with cross-linking Ab results in activation of Bruton’s tyrosine kinase and c-SRC, leading to the release of immune-suppression factors by DCs that suppressed NF-κB signaling (51).

Emerging evidence suggests that TIDCs have an impaired Ag-presentation capacity. For example, using the B.16 mouse melanoma tumor model, Stoitzner et al. (52) showed that, despite uptake of Ag, TIDCs (CD11c-sorted cells) cannot cross-present the Ag to OVA-specific CD8+ OT-I and CD4+ OT-II T cells. We used PD-1+ TIDCs from the ID8 mouse model of ovarian cancer and observed that blockade of PD-1 on these PD-1+ TIDCs that were pulsed with OVA enhanced their ability to activate OT-1 T cells (K. Karyampudi, P. Lamichhane, and K. Knutson, unpublished observations). This suggests that PD-1 expressed on TIDCs might act as a switch that regulates the Ag cross-presentation capacity of TIDCs. In contrast, Engeldardt et al. (27) showed that murine TIDCs (CD123+) ingest tumor-derived protein and present processed Ags to infiltrating tumor-specific T cells, even engaging in long-lived interactions with TIDCs; however, these interactions are non-productive. In addition to regulating Ag-presentation capacity, tumors and/or TME are known to play a role in modifying DC activity by inducing the upregulation of microRNAs in these cells. Min et al. (53) showed that multiple microRNAs, such as miR-16-1, miR-22, miR-155, and miR-503, are upregulated in bone marrow–derived DCs by mouse ovarian, melanoma, cervical, lung, and breast cancer cell lines. In the presence of tumor

FIGURE 1. TIDCs are central to tumor-immune interactions in the TME. DCs are recruited into the TME and induced to upregulate PD-1 and TIM-3. Interactions of PD-1 with PD-L1 in the TME blocks responsiveness to danger signals and prevents T cell activation through reduced Ag presentation and costimulation. Danger signals are also reduced as a result of high TIM-3 expression, which binds HMGB1. T cells are preferentially drawn to TIDCs as they enter the TME. In addition to the lack of appropriate activating signals, T cell responses are blocked by engagement of PD-1 by PD-L1 on the DC surface. Tregs are also induced by the TIDCs to establish a tolerogenic environment.
cells, DCs undergo apoptosis at higher rates compared with DCs that were cocultured with normal fibroblasts. Furthermore, it was observed that these microRNAs specifically targeted PI3K/AKT, MAPK, and apoptotic signaling pathways in DCs.

**TIDCs block adaptive immunity.** Immature and paralyzed TIDCs suppress both innate and adaptive immune effectors using a wide variety of mechanisms (Fig. 1). The ligand for PD-1, PD-L1, is constitutively expressed on activated DCs, and its expression is further upregulated by the TME (54). Studies by Curiel et al. (54) and Krempski et al. (37) showed that blockade of PD-L1 specifically on murine TIDCs resulted in a better capability of TIDCs to stimulate T cell activation. Krempski et al. (37) also showed that the TIDCs coexpress PD-1 and PD-L1, suggesting the possibility that suppression of T cell activation may be mediated by either of the molecule’s interactions with PD-L1 or PD-1 on the T cells. Using PD-L1–knockout T cells, Krempski et al. went on to show that TIDC-bound PD-L1 mediated direct immediate T cell suppression, as assessed in MLR, whereas DC-bound PD-1 paralyzed TIDCs, rendered them unresponsive to danger signals, and downregulated costimulatory molecules and cytokines. Thus, the suppressive nature of TIDCs mediated by PD-L1 appears to be constitutively present but is overcome by increased levels of costimulatory molecules and cytokines. This balance between inhibitory and activating potential is reminiscent of the killer phenotype of NK cells, whose activity is a balance of several inhibitory and activating receptors (55).

Consistent with the idea that the immune-activating potential of TIDCs is a balance between multiple inhibitory and activating molecules, DCs possess mechanisms other than just PD-L1 to block T cell activation. Liu et al. (56) reported that murine lung tumor cells release large amounts of PGE2 and TGF-β, which results in the conversion of immune-activating DCs into immune-suppressive DCs (CD11c<sup>low</sup>CD11b<sup>high</sup>I<sup>α<sub>1</sub></sup><sup>low</sup>). These DCs suppress T cell responses that are mediated, in part, through the upregulation of arginase I, which degrades arginine, an amino acid that T cells are unable to produce themselves and that is required for CD4 T cell proliferation. Higher arginase expression also may lead to higher levels of reactive oxygen intermediates, which block Ag-specific CD8 T cell responses (57).IDO expression by TIDCs also plays an important role in mediating the suppression of adaptive-immune responses (58). Tumor-derived PGE2 induces IDO expression in TIDCs, and these IDO<sup>+</sup>TIDCs suppress CD8 T cell responses to Ags presented by these TIDCs themselves, as well as those presented by third-party, nonsuppressive DCs. TIDCs also may suppress adaptive-immune responses indirectly. Indirect mechanisms include the induction of Tregs (by human pDCs) (59). Cytokines, such as TGF-β, IL-10, and IL-2, induce DCs to stimulate Treg formation in vitro (60). Recently, it was described that these cytokines may act together with cosignaling/surface molecules, such as inducible T cell costimulator ligand, PD-L1, CD80, and CD86, to induce DCs to stimulate Treg formation (59, 61–64).

The paralyzed, immune-suppressive phenotype of TIDCs is regulated by hyperactivation of tumor-induced transcription factors. TIDCs also were reported to overexpress several important regulatory genes that regulate their immune-suppressive and/or paralyzed phenotype. The most prominently studied is the transcription factor STAT3, which was found to be hyperactivated in DCs following exposure to tumor-derived factors (65, 66). STAT3 hyperactivation induces S100A9 protein, which prevents full maturation of DCs, thereby blocking their responsiveness to local danger signals (67). We also found that IL-10, which is overexpressed in the TME of various tumors, can activate STAT3 and induce enhanced expression of PD-1 and PD-L1 on DCs (K. Karyampudi, P. Lamichhane, and K. Knutson, unpublished observations), rendering them ineffective. Human and murine prostate cancer TIDCs (pDCs and non-pDCs) also were reported to overexpress another cell cycle–associated transcription factor, FOXO3. Its overexpression upregulates IDO, arginase, and TGF-β while diminishing the expression of costimulatory molecules and the release of inflammatory cytokines (43).

**Therapeutic manipulation of TIDCs**

Inroads have been made into the issue of how to modify TIDCs effectively. Their central role in the maintenance of innate- and adaptive-immune responses, as well as their ability to present Ag to effector cells, marks them as attractive targets for cancer treatment (68, 69). Over the past two decades, DCs have been used in vaccine models in preclinical and clinical settings. However, an intriguing new area of potential therapeutic impact is the manipulation of TIDCs. This has not been translated into the clinical setting, but several promising pathways and targets have been investigated recently.

Targeting the pathways that paralyze TIDCs appears to be a promising approach; certainly, one of the most attractive pathways is to specifically target the PD-1/PD-L1 inhibitory axis, particularly considering the rapid advances in the clinical development of biologics that block PD-1 and PD-L1 interactions (70). This may be particularly true with respect to advanced cancers in which PD-1 becomes highly expressed on TIDCs (37). Blocking the ligation of PD-1 on DCs, as was discussed above, restores danger responsiveness with increased T cell–activation capabilities (37). Furthermore, recent data from our group showed that PD-1 blockade, specifically on murine TIDCs, also leads to an increase in IL-7R expression by T cells, resulting in increased persistence in the TME (47). Further studies to validate the anti–PD-1–mediated rescue of immune-stimulatory capacities of TIDCs will open up pathways for combination therapies in the clinical setting that are aimed at enhancement of antitumor immunity via the TIDC compartment of the TME. Anti–PD-1 Ab is under investigation and showed some efficacy and safety in patients with different tumor types (71). It was approved recently by the U.S. Food and Drug Administration for use in patients with unresectable or metastatic melanoma if disease progresses following ipilimumab or in case of BRAF V600 mutation following BRAF-inhibitor treatment. Similarly, therapeutic biologics targeting the immune-suppressive molecule TIM-3 are under development and showing great promise (72). Given the multiple cell types (e.g., TIDCs, T cells, macrophages) in the tumor that express both PD-1 and TIM-3, it will be necessary to carefully craft translational studies that enable a better understanding of the impact of these therapeutic modalities on TIDCs. Alternatively, another potential modality is to block tumor-produced factors that induce TIDC paralysis, such as IL-10, one of the key cytokines known to upregulate both TIM-3 and PD-1 on TIDCs (50) (K. Karyampudi, P. Lamichhane, and
K. Knutson, unpublished observations). Vicari et al. (73) showed that TIDCs (most of which were of the immature phenotype) that were refractive to stimulation by a combination of LPS, anti-CD40, and IFN-γ could be rescued by treatment with a combination of CpG and anti–IL-10R Ab both in vitro and in vivo, leading to better de novo IL-12 production and antitumor immune responses.

Other studies aimed at inducing the maturation of TIDCs have been conducted. Saito et al. (74) showed that, when tumor-bearing mice are treated with plasmids containing cDNA for Flt3L or intratumoral injection with adenoviral vector carrying the IL-18 gene, the TIDCs expressed higher CD86 and exhibited better potential to activate T cells. Potent antitumor activity also developed in distant uninjectured tumors from the treated mice. In addition to this, given the prominent role of microRNAs in maintaining the function of TIDCs, delivery of modulators of microRNAs may be of crucial importance to the function of TIDCs in the TME with more immune-stimulatory DCs, indicating that the TIDC compartment in the TME is being renewed continuously.

Lastly, other interesting avenues for modification of TIDC behavior and phenotype are being explored. In one study of a murine model of lung cancer, a population of TIDCs was identified that increased in the tumor as the cancer progressed. Polarization of the TIDCs was found to be influenced by small rho GTPase signaling, which was reported previously as being integral to DC endocytosis, Ag processing/presentation, and motility. Rho GTPase inhibitor toxin B blocked the effect of paclitaxel in preventing a tumor-induced shift toward regulatory DCs, offering a possible mechanism for tumor immunologic escape and a future point of intervention (77). Lactic acid production by tumor cells may be another means by which cancer cells are able to induce immune-suppressive TIDCs. For example, in spheroid cultures of tumor cell lines, melanoma and prostate carcinoma cells were noted to produce high levels of lactic acid. The addition of lactic acid to in vitro–cultured DCs produced a phenotype similar to melanoma and prostate TIDCs (i.e., decreased IL-12 production and immature phenotype), and blockade of lactic acid reverted the phenotype to normal (78).

Targeting of these immune therapies to the TME in clinical practice will be a challenge, given the complexity of immune regulation; there are several approaches under investigation by which these pathways may be used. The development of a systemic Ab affecting a tolerogenic pathway, such as an anti–PD-1 Ab or biologic targeting of TIM-3, is one such method, and several agents are currently in development or under clinical investigation. Manipulation of immune tolerance and increased activation of DCs in combination with development of a DC-based vaccine for cancer Ag presentation would be another approach, as well as intratumoral delivery of agents or TIDC-modulating agents given in conjunction with chemotherapy to boost efficacy.

**Conclusions**

Over the past few years, the concept of the TME in the setting of malignancy has become well developed; although many immune cells play integral roles in the TME, it is increasingly clear that DCs are central mediators. The complexities of their role have yet to be fully elucidated, but studies have yielded promising insights into their function within the TME and directions for DC-targeted cancer therapy. Evident now is the fact that TIDCs are impaired in a variety of ways, which lead these DCs to confer immune suppression, rather than immune stimulation, at the local TME. Preclinical studies demonstrate promising areas for therapeutic intervention, such as the use of chemotherapeutics that work at lower, nontoxic doses for immune mobilization, blocking the pathways that induce tumor-induced shifts to a suppressive or regulatory DC phenotype, and targeted activation of TIDCs. Understanding the mechanisms and players involved in inducing such impaired and immune-suppressive DCs will open up avenues to explore therapies directed toward rescuing the immune-stimulatory potential of TIDCs.

**Disclosures**

The authors have no financial conflicts of interest.

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