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Review

Deciphering the Message Broadcast by Tumor-Infiltrating Dendritic Cells

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Human dendritic cells (DCs) infiltrate solid tumors, but this infiltration occurs in favorable and unfavorable disease prognoses. The statistical inference is that tumor-infiltrating DCs (TIDCs) play no conclusive role in predicting disease progression. This is remarkable because DCs are highly specialized antigen-presenting cells linking innate and adaptive immunity. DCs either boost the immune system (enhancing immunity) or dampen it (leading to tolerance). This dual effect explains the dual outcomes of cancer progression. The reverse functional characteristics of DCs depend on their maturation status. This review elaborates on the markers used to detect DCs in tumors. In many cases, the identification of DCs in human cancers relies on staining for S-100 and CD1a. These two markers are mainly expressed by Langerhans cells, which are one of several functionally different DC subsets. The activation status of DCs is based on the expression of CD83, DC-SIGN, and DC-LAMP, which are nonspecific markers of DC maturation. The detection of TIDCs has not kept pace with the increased knowledge about the identification of DC subsets and their maturation status. Therefore, it is difficult to draw a conclusion about the performance of DCs in tumors. We suggest a novel selection of markers to distinguish human DC subsets and maturation states. The use of these biomarkers will be of pivotal importance to scrutinize the prognostic significance of TIDCs. ***(Am J Pathol 2012, 181:733–742; http://dx.doi.org/10.1016/j.ajpath.2012.05.012)***

Cancer development follows six distinct hallmarks: the self-production of growth hormones, imperviousness to growth inhibitory signals, avoidance of apoptosis, unlimited proliferation, sustained angiogenesis, and metastatic capacity.[1] Recently, two emerging hallmarks have been added to the list: the reprogramming of energy metabolism and immunosurveillance evasion.[2] The importance of the latter hallmark is emphasized by a variety of opinions that cancers develop either to elude an antitumor immune response or to induce a tolerogenic response that facilitates tumor growth, as proposed by Dunn et al[3] and Zitvogel et al.[4] The tumor mechanisms that subvert the immune response include the inhibition of antigen presentation, the inhibition of tumor resident immune cells, and the active recruitment of suppressive immune cells.

Tumors are infiltrated by massive amounts of innate and adaptive immune cells, a feature that has long been observed and studied.[5] In addition, infiltrates of various types of immune cells may be correlated with disease prognosis, both positively and negatively.[5,6] The inclusion of immune cell infiltration as a routine measure of cancer prognosis has recently been reiterated by Pagès et al,[7] although their studies focused on cells of the adaptive arm of the immune response. The ability of these immune effector cells to execute either an (antitumor) immune response or a tolerogenic response, however, strongly depends on signals provided by antigen-presenting cells.

Dendritic cells (DCs) are the dominant professional antigen-presenting cells of the immune system; they are necessary for balancing tolerance and immunity.[8] DCs constitute a diverse family of cells that reside in various tissues and in the circulation. In addition, several studies have shown that DCs infiltrate a vast range of human tumors (Table 1).[9–52] Moreover, the presence of DCs has

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Table 1. DC Infiltration in Tumors in the Literature

Source	Population (No.)	Markers used	DC type	Location	DC state	Prognosis
Breast carcinoma tumors						
Bell et al[9]	32	CD1a, langerin, CD83, DC-LAMP, CD11c	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Mature	NA NA
Lespagnard et al[10]	143	S-100	Myeloid, LC like	NA	NA	No correlation
Hillenbrand et al[11]	52	CD1a, S-100	Myeloid, LC like	Intratumoral	NA	Good
Tsuge et al[12]	85	CD1a, S-100	Myeloid, LC like	Intratumoral Peritumoral	$CD1a^+$ $S\text{-}100^+$	NA NA
Coventry et al[13]	40	CD1a, CMRF-44, CMFR-56, CD83	Myeloid, LC like	Tumor stroma Around ductal formations	Immature	NA
Iwamoto et al[14]	130	CD1a, S-100, CD83	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Mature	No correlation Good
Coventry and Morton[15]	48	CD1a	Myeloid, LC like	Intratumoral	NA	Improved
Treilleux et al[16]	152	CD1a, langerin, DC-LAMP, CD123	Myeloid, LC like Myeloid, LC like Plasmacytoid	Intratumoral Peritumoral Intratumoral	Immature Mature Immature	No correlation No correlation Bad
Colorectal cancer tumors						
Ambe et al[17]	121	S-100	Myeloid, LC Like	Invasive margin Tumor center	NA	Good
Suzuki et al[18]	20	CD1a, CD83	Myeloid, LC like Myeloid, LC like	Tumor Invasive margin	Immature Mature	Unknown
Nakayama et al[19]	30	S-100	Myeloid, LC like	Tumor periphery	NA	Good
Dadabayev et al[20]	104	S-100	Myeloid, LC like	Invasive margin Tumor stroma Tumor epithelium	NA	No correlation
Sandel et al[21]	141	CD1a, S-100, DC-LAMP	Myeloid, LC like Myeloid, LC like	Invasive margin Tumor stroma Tumor epithelium	Immature Mature	Bad Bad
Nagorsen et al[22]	40	CD1a, S-100, langerin, CD11c, CD123, DC-LAMP	Myeloid, LC like	Tumor stroma Tumor epithelium	Immature	Good
Lung cancer tumors						
Nakajima et al[23]	90	S-100	Myeloid, LC like	Intratumoral	NA	Unknown/no
Zeid and Muller[24]	130	S-100	Myeloid, LC like	Intratumoral	NA	Good
Inoshima et al[25]	132	S-100	Myeloid, LC like	Intratumoral	NA	Good
Kurabayashi et al[26]	69	S-100	Myeloid, LC like	Intratumoral	NA	NA
Perrot et al[27]	22	CD11c, BDCA-2, CD83, Lin-	Myeloid, LC like Plasmacytoid	Intratumoral	Immature Immature	NA NA
Tabarkiewicz et al[28]	50	CD1c, CD19, CD123, BDCA-2	Myeloid Plasmacytoid	NA	NA NA	Bad No correlation
Melanoma tumors						
Vermi et al[29]	15	CD1a, langerin, DC-SIGN, CD206, BDCA-2	Myeloid, LC like Plasmacytoid	Intratumoral Peritumoral	Immature Immature	NA NA
Ladanyi et al[30]	82	CD1a, DC-LAMP	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Mature	Good Good
Ovarian carcinoma tumors						
Bethwaite et al[31]	73	S-100	Myeloid, LC like	Intratumoral	Immature	Good
Zou et al[32]	ND	Tumor depleted: CD3, CD14, CD16, CD19 and CD56 >pDCs isolated: $CD4^+CD11c^-$	Plasmacytoid Plasmacytoid	Intratumoral Peritumoral	Immature Immature	NA NA

(table continues)

Table 1. *Continued*

Source	Population (No.)	Markers used	DC type	Location	DC state	Prognosis
Wei et al[33]	ND	pDC sorted by $CD4^+CD123^+HLA\text{-}DRbrightCD11c-$	Plasmacytoid	NA	Immature	NA
Labidi-Galy et al[34]	40	Tumor depleted: CD3, CD8, CD11b, CD14, CD16, CD19, CD20, CD56, EPCAM and glycophorin A pDCs isolated: BDCA4-beads	Plasmacytoid	NA	Semimature	Bad
Renal cell carcinoma tumors						
Thurnher et al[35]	17	CD80, CD83, CD86 MHC class I and II, CD54	Myeloid, LC like	NA	Mature	NA
Troy et al[36]	14	CD1a, S-100, CD80, CD86, CD14, CD16, CMRF-44, CD83	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Mature	NA
Schwaab et al[37]	17	CD1a, CD40, CD80, CD83, CD86, CD54, MHC class II	Myeloid, LC like	Throughout Throughout	Immature Mature	No correlation No correlation
Aso et al[38]	33	CD83	Myeloid, LC like	Peritumoral	Mature	No correlation
Feng et al[39]	ND	CD1a, CD83	Myeloid, LC like	Intratumoral Peritumoral	Immature ($CD1a^+$) Mature ($CD83^+$)	No correlation Bad
Kobayashi et al[40]	142	S-100, CD83, MHC class II	Myeloid, LC like	Intratumoral	Immature ($S\text{-}100^+$) Mature ($CD83^+$)	Improved Good
Head and neck cancer tumors						
Kerrebijn et al[41]	18	CD1a, S-100, MHC class II, RFD1	Myeloid, LC like	Intratumoral Peritumoral	Mature ($CD1a^-$, $S\text{-}100^-$) Immature ($CD1a^+$, $S\text{-}100^+$)	NA NA
Goldman et al[42]	43	CD1a, S-100	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Immature ($CD1a^+$)	No correlation Good
Reichert et al[43]	132	S-100	Myeloid, LC like	Intratumoral	Immature	Good
Ishigami et al[44]	203	S-100	Myeloid, LC like	Intratumoral	Immature	Good
Hartmann et al[45]	16	BDCA-2, CD123, MHC class II	Plasmacytoid	Throughout	Immature	NA
Liu et al[46]	45	CD1a, S-100, DC-LAMP	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Immature Mature	NA NA
Bladder cancer tumors						
Inoue et al[47]	90	S-100, MHC class II	Myeloid, LC like	Intratumoral	Immature	Good
Ayari et al[48]	53	CD83	Myeloid, LC like	Intratumoral	Mature	Worse
Gastric cancer tumors						
Tsujitani et al[49]	210	S-100	Myeloid, LC like	Intratumoral	Immature	Good
Ishigami et al[50]	165	S-100, MHC class II	Myeloid, LC like	Intratumoral	Immature	No correlation
Takahashi et al[51]	ND	S-100	Myeloid, LC like	Intratumoral	Immature	Good
Ishigami et al[52]	128	S-100, DC-LAMP	Myeloid, LC like Myeloid, LC like	Intratumoral Peritumoral	Mature Mature	Bad Bad

NA, not available; ND, not determined.

been linked to a beneficial disease prognosis. Other studies have found an inverse correlation, doubting the value of infiltrating DCs. Our current knowledge of DC biology, however, shows that the markers historically used to detect DCs in tumors (S-100 and CD1a) are subset-specific rather than universal DC markers. Hence, the early investigations in the 1970s and some more recent studies have been biased by the availability and choice of DC-specific markers, respectively.

This review elaborates on the markers used to detect and characterize tumor-infiltrating DCs (TIDCs). We review the reported correlations between TIDC detection and disease prognosis. The statistical inference is that TIDCs play no conclusive role in predicting disease progression. Our current understanding of DC biology has increased considerably with respect to the tumor microenvironment and cancer immunotherapy. We suggest that the application of other DC subset-specific markers is pivotal for the acquisition of unambiguous information about TIDCs that could explain the association between TIDCs and cancer prognosis.

DCs Are First in Command when Regulating Immunity

DCs are the most potent professional antigen-presenting cells of the immune system. On infection or inflammation, immature DCs activate and differentiate into mature DCs. These mature DCs instruct the innate immune cells and select antigen-specific B and T lymphocytes, activating them to initiate adaptive immunity.[53] DCs sample and

process material from their microenvironment. They ingest proteins, break them down into peptides, and present them in major histocompatibility complex (MHC) molecules. The DCs are matured by danger signals derived from aberrant processes (eg, bacteria, viruses, apoptotic cells, and cancer peptides). After maturation, DCs migrate to the lymph nodes (LNs), where they present the peptides to T cells via MHC molecules. Recognition of the peptide-MHC complex (signal 1) and stimulation via costimulatory molecules (signal 2) and cytokines (signal 3) leads to the full-scale activation of immunocompetent effector T cells. The immunologic result strongly depends on the integration of these three signals. The resting DCs (ie, DCs receiving inhibitory signals, such as IL-10 or corticosteroids) provide only signal 1, leading to immune tolerance via T-cell anergy or the induction of regulatory T cells (Tregs). Fully matured DCs provide all three signals and induce immunity.

Despite their indispensable role in triggering an immune response, DCs are a rather rare and heterogeneous type of immune cell. DC subtypes differ in phenotype and function, which partly depend on their localization.[54] Langerhans cells (LCs) are DCs found predominantly in the epidermal layers of the skin, where they are primarily involved in the uptake and presentation of microbial antigens. This tissue-specific subset is characterized by the expression of langerin. Human peripheral blood contains two main subtypes of DCs: the CD11c-expressing myeloid DCs (mDCs) and the CD11c-negative plasmacytoid DCs (pDCs).[55] mDCs are further characterized by the expression of general myeloid markers, such as CD13 and CD33. They lack lineage-specific markers (CD3, CD14, CD19, and CD56), but they express high levels of MHC class II. Blood-residing mDCs are further subdivided according to their differential surface expression of CD1c (BDCA-1), CD16, and BDCA-3. All three mDC subsets have the capacity to produce IL-12 in response to microbial stimuli.[56–58] These mDC subsets, however, clearly differ in their expression of surface molecules and pattern recognition receptors and their potency to stimulate T cells.[8,59,60] CD16 mDCs were found to induce potent immune responses by their secretion of tumor necrosis factor-α, whereas BDCA-1 mDCs play a central role in recruiting other immune cells via IL-8 production. BDCA3 mDCs, however, were shown to efficiently cross-present exogenous antigen to cytotoxic T cells, a process that is essential for the induction of antitumor immune responses.[61] pDCs are distinctively identified by their expression of BDCA-4, BDCA-2, and CD123. They represent a highly specialized, naturally occurring DC subset that plays a major role in shaping innate and adaptive immune responses via the production of type I interferons.[62,63] pDCs promptly secrete large amounts of type I interferons in response to unmethylated CpG oligonucleotide motifs derived from bacterial and viral DNA/RNA, which they sense via the pattern recognition receptor Toll-like receptor 9.[64,65] pDC-derived type I interferons (eg, mDC-derived IL-12) participate in T-cell priming as T_H1-inducing cytokines.[66,67] Their heterogeneous phenotypes allow the different DC subsets to distinctly respond to the danger signals they encounter in their microenvironment. Hence, it is not merely the presence of a DC but the DC's specific subset type and maturation status that predicts the nature of the immune response.

The Prognostic Value of TIDCs

The observation that immune cells infiltrate tumor tissue suggests that the immune system plays a prominent role in tumor control. Early reports of beneficial infiltration have certainly stimulated research to clarify the role of immune cell infiltrates in tumors. Pathologists, oncologists, and immunologists have focused on DCs because they are the first cells to respond to antigens. The clinical significance of TIDCs has been reported for a variety of solid tumors, as evidenced by the correlations found between the presence of TIDCs and clinical prognosis (Table 1). However, the reported correlations range from positive to negative. The density and activation state of TIDCs can be determined by a variety of different molecules, but the most widely used are S-100/CD1a and CD83/DC-LAMP. In the case of breast carcinoma, Bell et al[9] demonstrated that immature DCs expressing CD1a and langerin are located throughout the tumor, whereas $CD83^+$ and $DC\text{-}LAMP^+$ (mature DCs) reside in the peritumoral areas. Several other studies have also found that immature TIDCs are preferentially located intratumorally, whereas mature TIDCs reside primarily in the peritumoral environment (Table 1). Note that although the different tumoral locations were not investigated in all studies, some studies do support the correlations reported by Bell et al.[9] Most studies of breast carcinoma could not find any clinical correlation between the infiltration of S-100+ and CD1a+ cells and prognosis.[9–16] Three studies report improved or positive prognostic values (CD1a, S-100, and CD83).[11,14,15] One recent study found that immature pDCs (CD123) predict an unfavorable prognosis.[16] Immunohistologic analyses of colorectal cancer samples similarly show that TIDCs can indicate either unfavorable[21] or favorable[17,19,22] prognoses. Moreover, the same discrepancies have been reported for lung cancer,[23–28] ovarian carcinoma,[31–34,68] bladder cancer,[47,48] and gastric cancer.[49–52] In contrast, overall positive clinical correlations between TIDCs and prognosis were found for melanoma[29,30] and head and neck cancer.[41–46]

Several studies report on the concomitant depletion of DCs in the blood in addition to their accumulation in tumors. The malignant ascites of patients with ovarian cancer have been found to be infiltrated by pDCs, whereas pDCs are depleted from the circulation.[69,70] These findings suggest an active recruitment of pDCs from the blood into the tumor by chemokines secreted in the tumor microenvironment (Figure 1). In 2001, Zou et al[32] reported that pDCs are recruited into ovarian cancer ascites by SDF-1. The depletion of circulating DCs in the blood has also been observed in patients with pancreatic cancer[71] and in patients with non–small-cell lung cancer.[28] These decreases correlate with poor patient survival.

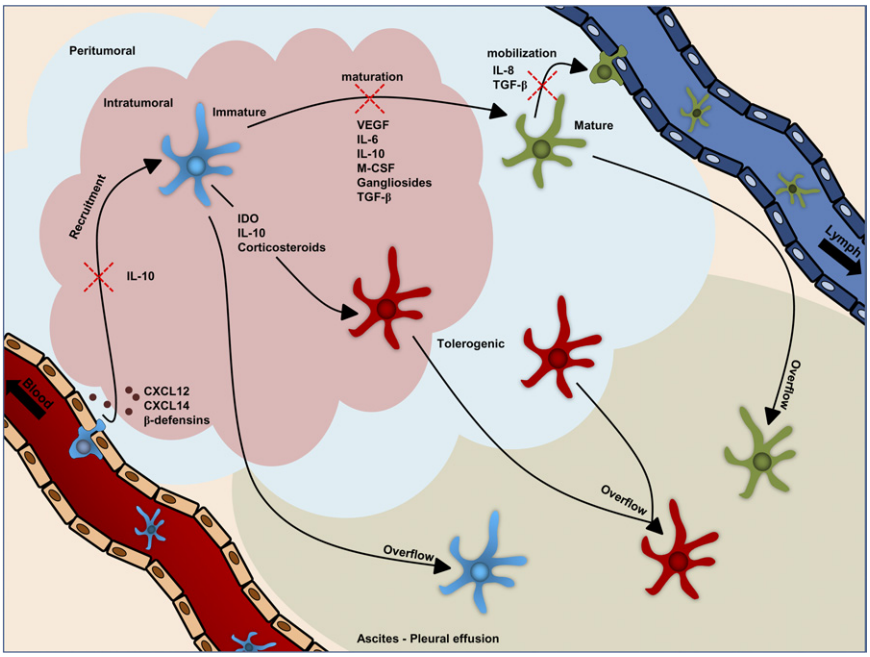

Figure 1. DCs are recruited into the tumor from the blood by different chemokines expressed or secreted in the tumor microenvironment. When present in the tumor, TIDCs can take up antigens, become activated, and migrate to the draining LNs to instruct T cells to mount antitumor immune responses. Several soluble factors and immune cells present in the tumor microenvironment can hamper the process induced by TIDCs. Therefore, it is important to understand which of these factors need to be targeted by specific therapy combined with TIDC activation. DCs are designated as immature (blue), mature (green), and tolerogenic (red). Factors that block recruitment, maturation, or mobilization are noted with a **red X** on the **arrow**. M-CSF, macrophage colony-stimulating factor; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

The general conclusion based on these studies is that TIDCs are evidently not correlated with disease progression. The various analyses, however, differ considerably in their use of markers to detect the presence of DCs and the discrimination of their activation status.

Histologic Markers for TIDC Characterization

The density, type, and activation state of TIDCs has been determined with a variety of different molecules, but S-100 and CD1a are the most widely used markers. However, after decades of research, the predictive significance of TIDCs detected using these markers remains unresolved.

S-100 is a small, acidic regulatory protein involved in a wide range of cellular processes. In the early 1980s, Cocchia et al[72] reported that this protein is expressed by LCs. Concomitantly, mature interdigitating DCs in the LNs were shown to express S-100.[73] Shortly after these findings were published, S-100$^+$ dendritic-like cells were found to be present in various primary and metastatic tumors (Table 1). Moreover, for a long time, the S-100 protein was the only marker available for the evaluation of TIDCs in tumors. The surface molecule CD1a was later added to the armamentarium for TIDC study. Similar to S-100, CD1a expression was originally attributed to LCs.[74] Later studies demonstrated that interstitial DCs in the dermis and *in vitro* cultured monocyte-derived DCs also express CD1a molecules[75] but that none of the blood DC subsets express S-100 or CD1a. Among the tissue-resident and peripheral DCs, the expression of CD1a and S-100 is restricted to LCs or to LCs and interdigitating DCs. In addition to the constrained specificity of these markers for only a few DC subsets, the S-100 protein is also expressed by macrophages.[74] Despite these limitations, S-100 and CD1a are the markers most commonly used to detect DC infiltrates in tumors.

In addition to the restrictions on subset identification, the studies based on S-100 and/or CD1a expression could not determine the functional status of the TIDCs. Although CD1a was initially described as a marker for immature DCs, different *in vitro* and *in vivo* studies have shown that CD1a and S-100 are present in mature and immature cells.[9,15,76] Successive studies have endeavored to assess the maturation status of TIDCs by staining for CD83, a DC-specific maturation marker expressed by all subsets.[77,78] Recent investigations have focused not only on the presence of TIDCs but also on their activation status. The discovery of such molecules as DC-LAMP and DC-SIGN has also garnered interest in the characterization of TIDCs.[79,80] DC-SIGN is expressed by immature DCs, whereas DC-LAMP is present on mature DCs. These markers could improve the ability to distinguish the activation state of TIDCs as a first step toward describing their functional status and understanding their potential immunomodulatory role. Current knowledge shows that DC-SIGN is expressed by LCs and other tissue-resident DCs but not by any of the blood DC subsets, indicating a confined characterization of TIDCs.

The identification of several naturally occurring DC subsets has prompted studies aimed at specifically detecting the infiltration of tumors by the distinct subsets.[81] Consequently, pDCs were found to infiltrate melanomas,[29] ovarian carcinoma and its ascites,[32,34] lung cancer,[27,28] head and neck cancer,[45] and breast carcinomas.[16] These studies were mainly based on the expression of BDCA-2 and/or CD123. However, CD123 is not exclusively expressed by pDCs and, thus, requires costaining with BDCA-2, or BDCA-4 for pDC identification. Moreover, BDCA-2 is down-regulated on pDC maturation.[82] Hence, studies using this antibody will most

likely identify only immature tumor-infiltrating pDCs. In addition to pDCs, BDCA-1$^+$ mDCs have also been demonstrated to infiltrate solid tumors, as demonstrated by their presence in non–small-cell lung cancer tissue.[28]

In summary, numerous studies have attempted to characterize TIDCs; however, because the studies lacked extensive analysis, it is impossible to tell whether those TIDCs (immature and especially mature) were LCs, interstitial DCs, mDCs, or pDCs. Based on the TIDC analysis, no valid conclusion about their role in tumorigenesis can be drawn. However, the current situation for TIDCs is remarkably similar to past reports for tumor-infiltrating T cells. Based on improvements in the characterization of several T-cell subsets, a better correlation between DCs and progression has been envisaged. The discrimination of CD4-positive cells in conventional CD4 T cells and Tregs was found to be predictive in several tumors types (particularly brain and ovarian cancer).[70,83]

We propose a similar approach for TIDCs and suggest that DC subset and maturation status markers will enable valid conclusions to be drawn about the correlations between tumor infiltration and disease outcome. To find the required markers, one must learn about the functional characteristics of TIDCs.

TIDC Functionality

DC tumor infiltration reflects the host system's response to signals provided by the tumor and its microenvironment. TIDCs are thought to capture and process antigens that are released in the tumor microenvironment and then migrate to draining LNs, where they may mount an antitumor immune response (Figure 1). In mouse models, however, effective immune responses and the specific activation of naive T cells have also been reported directly in the tumor.[84] In their study, Thompson et al[84] examined antitumor responses when T-cell migration from the LNs is blocked chemically. They also examined mice with a complete absence of LNs. The activation of naive T cells in the tumor is at least partially mediated by tumor-resident antigen-presenting cells.

In many instances, soluble factors secreted by the tumor and its microenvironment have been reported to create an immunosuppressive environment, thereby preventing the differentiation and maturation of infiltrating immune cells. Tumors actively recruit various immunosuppressive cells, including Tregs, myeloid-derived suppressor cells, and tumor-associated macrophages (TAMs).[85] In response to soluble factors secreted by the tumor, these cells secrete suppressive molecules that recruit additional tolerogenic immune cells and inhibit the maturation and differentiation of tumor-residing naive cells, such as TIDCs (Figure 1).[9,86,87] The role and significance of soluble factors that influence TIDC function and lead to tumor growth and progression have been described extensively in excellent reviews[88,89]; therefore, these issues are only briefly addressed herein. Immunosuppressive factors can be expressed by malignant cells and/or other cells present at the tumor site, such as immune, epithelial, or stromal cells. One of the most prominent factors is most likely transforming growth factor β, a cytokine that affects the proliferation, activation, and differentiation of immune cells and inhibits antitumor immune responses (Figure 1). IL-10 is another immunosuppressive molecule that has been shown to negatively affect TIDC maturation and TIDC recruitment to the tumor; moreover, it correlates positively with the generation of tolerogenic TIDCs (Figure 1).[89]

Two recent articles effectively illustrate the importance of the maturation status of immune cell that have penetrated the tumor. The infiltration of breast cancer by immature TAMs was shown to be predictive of reduced progression-free survival and overall survival. Accordingly, the chemical inhibition of TAM infiltration into mammary carcinomas slows the growth of primary tumors and decreases metastasis in mice.[90] In contrast, Beatty et al[91] showed that the activation of infiltrating macrophages by CD40 cross-linkage induces tumor regression in a mouse model of pancreatic carcinoma. CD40L (CD154) is primarily expressed by activated T cells; however, the T cells in pancreatic cancer tissue apparently fail to express CD154. Therefore, the underlying mechanism is most likely via DCs, which are the best T-cell activators in the immune system but are unable to induce the full activation of T cells. These studies indicate that the influx of immune cells that remain immature in the tumor microenvironment may even have a deleterious effect on patient survival.

The direct immunosuppressive character of the tumor microenvironment and the established clinical significance of such inhibitory immune cells as Tregs and TAMs stress the importance of an elaborate characterization of TIDCs in terms of subset allocation and maturation status.

Discriminating TIDC Characterization

Numerous studies have strived to characterize the tumor infiltration by DCs to understand their role in the tumor microenvironment. Thus far, the classical markers, namely, CD1a, S-100, CD83, and DC-SIGN, used for TIDC identification do not reveal unambiguous correlations with cancer progression. In the case of T cells, the discovery of immune suppressive and activating subsets and their specific detection in tumor samples provides the field with explicit correlations. These data stress the importance of the implications of T-cell infiltration for an accurate prognosis.[92] Moreover, this study shows the importance of intratumoral and peritumoral immune cell infiltration, both of which contribute to the prognosis. In this light, we advocate the use of a more appropriate set of markers to unequivocally determine the DC subset and maturation status (Table 2).

Expression of the historical markers S-100 and CD1a is restricted to only a few DC subsets. These molecules can be used in conjunction to detect CD1a$^+$, S-100$^-$ tissue-resident interstitial DCs. Interdigitating DCs can be characterized by the coexpression of S-100 and CD11c. Additional staining with CD11c is necessary to distinguish S-100$^+$, CD11c$^+$, CD1a$^-$ interdigitating DCs from TAMs that are S-100$^+$, CD11c$^-$, and CD1a$^-$. LCs can be read-

Table 2. Markers for DC Characterization

Marker	LC	inDC	IDC	BDCA-1$^+$ mDC	BDCA-3$^+$ mDC	pDC	Other immune cells
Langerin	I/M	–	–	–	–	–	–
CD1a	I/M	I/M	–	–	–	–	–
S-100	I/M	–	I/M	–	–	–	+
CD11c	I/M	I/M	I/M	I/M	I/M	–	+
BDCA-1	I/M	–	–	I/M	–	–	+
BDCA-3	–	–	–	M	I/M	M	–
BDCA-2	–	–	–	–	–	I	–
BDCA-4	–	–	–	M	–	I/M	+
CD123	–	–	–	I/M^{dim}	–	I/M	+
BAD-LAMP	–	–	–	–	–	I	–*
DC-SIGN	I	I	–	–	–	–	+
DC-LAMP	M	M	M	M	M	M	–
CD83	M	M	M	M	M	M	+

Proposed markers for unequivocal DC characterization are shown in bold.
*BAD-LAMP is expressed by brain tissue.
I, immature; IDC, interdigitating DC; inDC, interstitial DC; M, mature.

ily detected by their expression of langerin. The maturation status of these tissue-resident DC subsets can be determined by costaining with the activation marker CD83 or DC-LAMP.

Blood DC subsets can be identified with several available markers. The molecules BDCA-3 and BDCA-1 represent good candidate markers for the analysis of their respective mDC subsets. Because BDCA-1 is also expressed by B cells and LCs, additional markers (S-100 and CD11c) are needed to explicitly identify BDCA1$^+$, CD11c$^+$, and S-100$^-$ mDCs. Costaining with CD83 or DC-LAMP can be used to detect the maturation status of these cells. The identification of activated BDCA-3$^+$ mDCs is more complex because this molecule becomes up-regulated on the other matured blood DC subsets. Hence, the BDCA3$^+$, CD83$^+$ (DC-LAMP$^+$) cells must be negative for BDCA-1 and CD123. Immature pDCs can be readily detected by their expression of BDCA-2. Recently, the cytoplasmic protein BAD-LAMP has been reported to be specifically expressed by immature human pDCs, representing a suitable new marker.[93] Importantly, this protein is also expressed in brain tissue. BDCA-2 and BAD-LAMP are both down-regulated when pDCs mature. Thus, the active cells should be identified as CD123$^+$, CD11c$^-$, CD83$^+$(DC-LAMP$^+$).

The suggested marker set for identifying tumor subsets and maturation status is based on its appearance in healthy individuals. It is plausible that the tumor microenvironment will disturb the normal developmental program of a DC. Such putative aberrant expression can be deduced by testing their function after DCs are isolated from the tumor tissue.

Future Perspective of the Activation of TIDCs to Boost Antitumor Immunotherapy

Any type of immunotherapy aimed at tumor eradication will be greatly influenced by tumor-resident and circulating (draining LN) cells and factors. Therefore, cell-based therapies should be combined with inhibitory treatments of the immunosuppressive tumor microenvironment, which should be monitored carefully. Therapies that modulate Tregs have been shown to improve DC vaccination protocols.[94,95] Furthermore, data from murine and human studies demonstrate that intratumoral injections of activated DCs act synergistically with DC vaccines delivered in the periphery.[96] These data indicate that reactivation of tumor-resident DCs could boost peripheral vaccines.

Despite the ability of tumor-infiltrating pDCs to infiltrate solid tumors, the immunosuppressive environment disables their ability to sense released DNA/RNA via Toll-like receptors.[97–99] Therefore, the tumor-infiltrating pDCs remain in an inactivated state. These findings correlate with poor prognosis[16] and are linked to the induction of Tregs.[39] Several studies have now demonstrated that topical treatment with imiquimod (a Toll-like receptor 7 agonist) leads to enhanced pDC recruitment and type I interferon production by resident pDCs at the tumor site, thereby generating an inflammatory environment that results in tumor regression.[100,101] The intratumoral injection of CpG motifs (inducing Toll-like receptor 9 triggering) also has therapeutic potential in patients with basal cell carcinoma and melanoma skin metastases.[102] Another recent study of prostate cancer describes the presence of tolerogenic human TIDCs, which induce suppressive tumor-specific T cells. These TIDCs show elevated levels of FOXO3 that coincide with suppressive genes that negatively regulate T-cell function. The silencing of FOXO3 abrogates the ability of TIDC to induce suppressive activity by T cells. Moreover, in mice, this response also leads to the reduced expression of tolerogenic mediators, such as indoleamine 2,3-dioxygenase and transforming growth factor β, and to the enhanced expression of costimulatory molecules and proinflammatory cytokines.[103] Rather than reactivating TIDCs, this strategy focuses on reversing the balance by silencing immunosuppressive regulators in TIDCs. Future research should focus on the combination of the two different strategies, as they most likely act synergistically.

Conclusions

In recent decades, tremendous efforts have been made to understand the tumor microenvironment and the role of

TIDCs in tumor progression. Thus far, the detection of TIDCs does not unambiguously correlate with clinical parameters. To enable therapeutic strategies aimed at manipulating TIDCs, it is necessary to fully understand their functional state and to know which of the DC subsets are present in the tumor environment and where they are located. To obtain specific data about the presence, localization, function, and, ultimately, role of different TIDCs, the repertoire of surface markers that is used to distinguish between TIDC subsets and TIDC activation needs to be revised and updated. We anticipate that this specific information, coupled with clinical data, will provide us with a detailed roadmap that predicts disease progression and therapeutic success based on the presence, localization, and maturation status of TIDCs.

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