Immunosuppressive Mechanisms of Regulatory Dendritic Cells in Cancer

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Abstract Three major functional subsets of dendritic cells (DCs) have been described in the tumor microenvironment in patients with cancer and tumor-bearing animals: (i) conventional DCs with intact antigen-presenting capabilities, (ii) functionally defective DCs with decreased motility and low ability to uptake, process and present antigens or produce cytokines and (iii) regulatory DCs with high capacity to suppress T cell proliferation, induce differentiation of regulatory T cells or support immune tolerance. Phenotypic characteristics of regulatory DCs (regDCs), as well as the mechanisms of T cell inhibition, vary in different experimental conditions and environments, suggesting high level of their plasticity and probably different origin. Although new data demonstrate that regDCs may play an important role at early stages of tumor development, functional differences and clinical significance of emergence of different myeloid regulatory cells (MDSCs, regDCs, M2 macrophages, N2 neutrophils, mast cells) in cancer remain to be determined.

Keywords Dendritic cells · Tolerogenic dendritic cells · Immunosuppression · Regulatory dendritic cells · Immunosuppressive cytokines

Introduction: Regulatory Dendritic Cells in Cancer

Recently, a subset of dendritic cells (DCs) with the regulatory properties has been shown to actively and efficiently inhibit T cell response in the tumor microenvironment. It was also suggested that these immunosuppressive DCs may be involved in tumor escape from immunological destruction [1, 2]. In various conditions, such as transplantation,

allergy, autoimmune and infectious diseases, regulatory DCs (regDCs) have been described with different phenotypic characteristics [2–9]. Although Enk et al. (1997) reported that melanoma-derived factors converted the antigen-presenting function of conventional cDCs to tolerance induction against the tumor tissue [10], the mechanisms of how the tumor microenvironment programs the generation and differentiation of regDCs remain to be fully understood.

The stromal microenvironment of the spleen, lung and liver can induce appearance of regDCs with different phenotypes [11, 12]. For instance, murine lung cancer could “educate” cDCs to differentiate into $CD11c^{low}CD11b^{high}Ia^{low}$ regDCs that inhibit T cell response, as was reported by Liu et al. using the freshly isolated tumor cells to mimic the tumor microenvironment in DC co-culture studies [13]. Norian et al. found that tumor-infiltrating DCs could act as $MHCII^{+}CD11b^{+}CD11c^{high}$ regDCs by inhibiting $CD8^{+}$ T cell function in an L-arginine-dependent manner, resulting in diminished T cell-based anti-tumor immunity in vivo [14]. An altered cell fate program characterized by minimal T cell expansion, impaired $IFN-\gamma$ production, and anergy is resulted from the stimulation of naïve T cells with these regDCs. Tumor cell-induced conversion of immature myeloid DCs into TGF- β -secreting cells, which can induce $CD4^{+}CD25^{+}$ Treg cell generation, has been also reported [15, 16]. Interestingly, human lung carcinoma cells could also convert mature DCs into cells producing TGF- β 1. These TGF- β 1-producing DCs were poor at eliciting the activation of naïve $CD4^{+}$ T cells and sustaining their proliferation and differentiation, but revealed an increased ability to induce differentiation of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells [17]. Tumor-derived factors can convert/polarize conventional cDC into $CD11c^{low}CD11b^{high}$ regDCs that were able to actively suppress proliferation of pre-activated T cells [2].

Although a growing body of evidence demonstrates that regDCs play an important immunosuppressive and protumorigenic role in cancer, diversity of their phenotypic and functional appearance limits our understanding of their

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origin, differentiation pathways, biological significance and interaction with other immune regulatory cells. However, revealing the mechanisms of immunosuppressive and tolerogenic activities of regDCs may help enlightening similarities and differences between myeloid regulatory cell subsets and thus understand their specific roles in forming unique properties of the tumor immunoenvironment.

Characteristics of Tumor-induced Regulatory Dendritic Cells

Regulatory DCs are often referred to as tolerogenic DCs, which is often not correct if their tolerogenic properties are not confirmed or tested [18]. Other terms for regDCs are alternatively activated or suppressor or type II DCs; however these terms are seldom used. Regulatory DCs belong to the family of regulatory myeloid cells, which also include alternatively-activated macrophages or M2 macrophages, myeloid-derived suppressor cells (MDSCs), alternatively activated or N2 neutrophils and a subset of mast cells [1, 19]. Regulatory DCs are differentiated mature or immature CD11c+DCs that, however, do not yet have a specific marker similar to FoxP3 expression by regulatory T cells, which identifies them as immunosuppressive cells. Rather, regDCs can be identified using a combination of surface marker expression, cytokine production, immunomodulatory molecule expression and functional regulatory or suppressive properties.

Immunoregulatory molecules and markers, including PD-L1, PD-L2, B7-H3, B7-H4, CD103 and ILT3/4, increased production of immunoregulatory cytokines and factors (e.g., IL-10, IL-1 β , TGF- β , indoleamine 2,3-dioxygenase (IDO), arginase I, iNOS) or decreased production of pro-inflammatory cytokines (e.g., IL-6, IL-12, IL-15, TNF- α , IL-1 α) have been described in different regDC model systems [1, 20–25]. Plasmacytoid regDCs might express CCR9 [26]; in cancer, plasmacytoid regDCs may be identified as cells expressing FOXO3 [27]. In addition, tumor-induced regDCs have been described as DCs expressing low levels of CD11c, MHC class II and co-stimulatory CD80 and CD86 molecules and high levels of CD11b [13, 19]. Overall, both phenotypic and functional characteristics of reported regDC subsets are markedly different in various experimental conditions and model systems, suggestion not only the plasticity of their differentiation and dependence on the local microenvironment, but potentially the different origin of functionally immunosuppressive or tolerogenic regDCs. Although tumor-associated regDCs were recently reported in different tumor models, the mechanisms of their immunosuppressor activity are vary and might be tumor-specific. Much more studies are required for comprehensive

identification and characterization of regDCs in the tumor microenvironment.

Immunosuppressive and Tolerogenic Activity of Regulatory Dendritic Cells

Immune suppression induced by regulatory DCs can be mediated by a number of suppressive factors, such as catabolic enzymes IDO, which targets tryptophan, arginase, which utilizes L-arginine, iNOS, responsible for Nitric oxide (NO) production. RegDCs can also produce cytokines, such as IL-10 or TGF- β that directly suppress activity of effector T cells or alter polarization of T helper cells. Tumor-induced regDCs could inhibit the proliferation of T cells not only through TGF- β 1, but also by inducing the production of Treg cells or T cell anergy [28, 29]. Expression of inhibitory molecules on regDCs, like programmed cell death 1 ligand 1 (PD-L1) and PD-L2, is another pathway responsible for regDC-mediated inhibition of anti-tumor immunity. Although these and other mechanisms of immunosuppression induced by regDCs have been reported [14, 30–34], it is still unknown which tumor-derived factors or local microenvironmental stimuli are responsible for polarization of cDCs into regDCs in different model systems and for differential expression of a variety of immunosuppressive molecules in regDCs in different conditions. Interestingly, Kuang et al. have recently reported that hyaluronan fragments derived from cancer cells drove human monocytes to become tolerogenic semimature DCs, which triggered rapid down-regulation of CD3- ϵ and TCR- α/β and subsequent apoptosis in autologous T cells [35]. Regulatory DC-induced effect on T cells was markedly reduced by blocking of NADPH oxidase but not by inhibition of arginase, inducible NO synthase (iNOS), IDO, or IFN- γ [35]. Consistent with these results, accumulation of immunosuppressive DCs coincided with CD3- ϵ down-regulation and T cell deletion in cancer nests of human tumors.

Immunosuppressive Cytokines: TGF- β

Transforming growth factor beta (TGF- β) is involved in regulating many cellular processes in both the adult organism and the developing embryo including cell growth, cell differentiation, apoptosis, cellular homeostasis and other cellular functions. Many advanced tumors produce excessive amounts of TGF- β , which, in normal epithelial cells, is a potent growth inhibitor. However, in oncogenically activated cells, the homeostatic action of TGF- β is often diverted along alternative pathways. Hence, TGF- β signaling elicits protective or tumor suppressive effects during the early growth-sensitive stages of tumorigenesis. However, later in tumor development when carcinoma cells become

refractory to TGF- β -mediated growth inhibition, the tumor cell responds by stimulating pathways with tumor progressing effects [36].

Furthermore, TGF- β has pleiotropic effects on the immune system, and is recognized as one of the most potent immunosuppressive agents in facilitating oncogenesis. The TGF- β pathway promotes cancer progression by inhibiting the protective host immunity in both the innate and the adaptive arms of the immune response. Several mechanisms through which TGF- β interferes with the development of an anti-tumor immunity have been recognized [37]. For instance, TGF- β promotes tumor-associated macrophage polarization to an M2-versus-M1 phenotype, which further promotes TGF- β production and deepens immunosuppression [38]. TGF- β also inhibits the cytolytic activity of NK cells expressing the activating receptor NKG2D, further resulting in a poor antitumor response [39]. In the adaptive immune response, TGF- β promotes CD4⁺ T cell differentiation into Th2 cells rather than Th1 cells lessening the efficacy of anti-tumor immune response [40]. In the tumor microenvironment, TGF- β can induce T cells apoptosis [41]. TGF- β also inhibits the CD8⁺ T cells anti-tumor activity by suppressing the expression of several cytolytic genes, including the genes encoding granzyme A, granzyme B, IFN- γ and FAS ligand [42]. In addition to its direct anti-inflammatory effects on T cells, TGF- β has been implicated as central regulator of regulatory T cells. TGF- β not only mediates the suppression of effector T cells by Tregs, recent evidence also reveals a role for TGF- β along with TCR stimulation in the peripheral induction of regulatory T cells from naive CD4⁺CD25⁻ cells [43].

In support of the concept that certain DC subpopulations play crucial roles in tumor escape, it was recently reported that tumor expansion could stimulate Treg cells via a specific DC subset: During tumor progression, a subset of DC exhibiting a myeloid immature phenotype may be recruited to draining lymph nodes and selectively promote proliferation of Treg cells in a TGF- β -dependent manner [15]. Importantly, tumor cells are necessary and sufficient to convert DC into regulatory cells that secrete TGF- β and stimulate Treg cell proliferation.

Interestingly, as plasmacytoid DCs exert both proinflammatory and regulatory functions, TGF- β can be also involved in their immune-mediating activity in the tumor milieu. Recent data on pDC and TGF- β interactions revealed that as with many cell types, pDCs are able to respond to TGF- β using the classic Smad signaling pathway. In addition, pDCs are capable to secrete TGF- β , in particular in response to TGF- β exposure. Exposure of pDCs to TGF- β prevents type I interferon secretion in response to TLR7/9 ligands, while in contrast, the consequences of TGF- β on the antigen-presenting cell capacities of pDC are less clear, since TGF- β -exposed pDCs may lead to both regulatory T cell

and interleukin-17-secreting cell polarization [44]. In addition, TGF- β decreases cDCs migration and increases apoptosis, which decreases antigen presentation and dampens the adaptive immune response [45, 46].

Thus, a subset of regulatory TGF- β -secreting DCs in cancer may directly and indirectly maintain antigen-specific and non-specific T cell unresponsiveness by controlling T cell polarization, MDSC and Treg differentiation and activity, and affecting specific microenvironmental conditions in premalignant niches [1].

Immunosuppressive Cytokines: IL-10

IL-10 is another cytokine with pleiotropic effects in immunoregulation and inflammation commonly seen in the tumor milieu. It can be produced by cancerous cells, TAM, CD8⁺ T cells, DCs and is commonly regarded as an anti-inflammatory, immunosuppressive cytokine that favors tumor escape from immune surveillance. Similar to TGF- β , tumor-derived IL-10 can polarize DCs to regulatory phenotype, as well as acts in an autocrine circuit. For instance, in the tumor microenvironment, IL-10 may prevent maturation of DCs, decrease their ability to present tumor-associated antigens, reduce expression of MHC class I and II molecules and costimulatory molecules and attenuate production of inflammatory cytokines [38, 47–49]. IL-10 can also inhibit the release of IFN- γ and IL-2 and thus inhibit T cell growth [50]. This immunosuppressive effect of IL-10 is mediated by blocking the CD28 and ICOS costimulatory signaling [51, 52].

IL-10 is a key soluble molecule that critically contributes to immune tolerance by modulating DC costimulation. IL-10/IL-10R-signaling is mediated by activation of the Jak/STAT pathway through tyrosine phosphorylation of Tyk2/Jak1, resulting in downstream activation and homodimerization of STAT3. In the context of DC maturation by several stimuli the most important pathways affected by IL-10 are PI3K/Akt/NF- κ B-, MyD88/MAPK-, and Ras/Raf/MAPK-regulated signal transduction cascades [53]. Maturation of DC in the presence of IL-10 induces two distinct subsets of tolerogenic DCs characterized in humans as CD83^{high}CD80/CD86^{high}HLA-DR^{high} and as CD83^{low}CD80/CD86^{low}HLA-DR^{low}. Expression of surface molecules associated with DC maturation like CD83, the lymph node homing receptor CCR7 and MHC class-II molecules is reduced on IL-10-induced regDCs. In contrast, expression of inhibitory receptors such as immunoregulatory receptors like ILT2, ILT3, and ILT4 are increased on IL-10-induced regDCs, which has been suggested to be involved in tolerance induction [53]. The amount of proinflammatory cytokines released by IL-10-induced regDCs, like IL-6 and TNF- α , is similar to classically matured DCs. In contrast, IL-10 production is dramatically increased suggesting another possible mechanism resulting in tolerance

induction [54–56]. Several studies showed that in vitro generated tolerogenic IL-10-DCs have an even higher capacity to induce iTreg than tissue resident immature DCs [53]. Thus, a reduced expression of immunostimulatory molecules combined with a higher expression of inhibitory costimulators and the production of IL-10 may facilitate tolerogenic functions of IL-10-induced regDCs.

The tolerogenic function of IL-10-induced regDCs was demonstrated by induction of antigen-specific anergy in CD4⁺ and CD8⁺T cells [57]. This anergic state is characterized by low T cell proliferation and decreased IL-2 and IFN- γ secretion [58]. In addition, IL-10 DC-primed anergic CD4⁺ and CD8⁺T cells exhibit suppressive capacity and are able to inhibit activated Th1, Th2, and Tc1 T cell responses [29]. Compared to tolerogenic tissue resident DCs, IL-10-induced regDCs are terminally differentiated and exhibit a stable phenotype [53] and have been confirmed in peripheral blood of humans [55].

Immunosuppressive Cytokines: Other Molecules

Additional factors produced by regulatory DCs may also directly or indirectly support tumor growth through several pathways. For instance, in breast cancer, DCs could prime CD4 T cells to produce IL-13, which in turn promotes early tumor development and tumor growth [59]. Several studies have found that chemokines also play an important role in immunosuppression. Tolerogenic IL-10-induced DCs are characterized by enhanced production of CCL18, and CCL18 has the ability to recruit naïve T cells, which induces T cell anergy. Interestingly, differentiation of monocytes from healthy subjects in the presence of GM-CSF and CCL18 led to the development of DCs with a semimature phenotype, intermediate levels of co-stimulatory and MHC class II molecules, increased CCR7 expression, which induced IL-10-expressing type 1 T regulatory cells [60]. The generation of regDCs by CCL18 was dependent on the production of indoleamine 2,3-dioxygenase (IDO) through an IL-10-mediated mechanism. TGF- β 1 has been also reported to induce a significant increase in IL-10, impaired IL-12 secretion, and attenuated mRNA expression of chemokines CCL2, CCL3, and CXCL10 in DCs [61]. Furthermore, CCL-2 and CCL-5, which are chemoattractants of monocytes and DCs to tumors, induce suppression of T cell responses [62] and tumors recruit pre-cDCs from the circulation through a CCL3-dependent mechanism [63].

Additional Tolerogenic Pathways in regDCs: Arginase 1

Several years ago, tumor-infiltrating regulatory DCs were reported to inhibit CD8⁺ T cell function via L-arginine metabolism [14]. The authors were working on the mechanism by which tumor-associated DCs suppressed T cell

proliferation and revealed that tumor-associated DCs incorporated five times more L-arginine than did control DCs. L-arginine is metabolized into L-citrulline and NO by nitric oxide synthase NOS2 or it is metabolized into polyamine and urea by arginase 1. Norian et al. showed that culture of tumor-associated DCs led to increased arginase I protein expression and arginase activity, while NO production was not elevated [14]. Furthermore, use of iNOS inhibitors did not significantly increase T cell proliferation, while the addition of L-arginine to prevent its depletion from culture medium or an arginase inhibitor restored T cell proliferation to the levels that seen for T cells cultured with control DCs [14]. Taken together, these data indicate that tumor-associated DCs may inhibit T cells via arginase-mediated metabolism of L-arginine.

Increased arginase activity in tumor-associated regDCs has been reported for different cancer types and tumor models. The tumor microenvironmental conditions associated with low nutrient supply, hypoxia, low extracellular pH, and defects in glycoprotein and lipid biosynthesis, might induce stress of the endoplasmic reticulum (ER) in tumor cells. Eukaryotic cells cope with ER stress by engaging a conserved set of intracellular signaling pathways known collectively as the unfolded protein response (UPR). UPR signaling pathways are activated in primary solid tumors of diverse histological origin, but not in peritumoral areas, and ablation of UPR elements prevents tumor initiation or significantly decreases tumor growth, survival, and angiogenesis [64]. Interestingly, the tumor can function in a cell-extrinsic manner via the transmission of ER stress to DCs. Tumor UPR-treated DCs upregulated expression of arginase and pro-inflammatory tumorigenic cytokines; they also downregulated cross-presentation of antigen, fail to effectively cross-prime CD8⁺ T cells causing T cell activation without proliferation and dominantly suppress cross-priming by bystander DCs [64], suggesting the appearance of regulatory phenotype. In another study, using the freshly isolated tumor cells to mimic the tumor microenvironment, the authors found that tumor cells could drive DCs to differentiate into regDCs with a CD11c^{low}CD11b^{high}Ia^{low} phenotype and high expression of IL-10, NO, vascular endothelial growth factor (VEGF) and arginase I [13]. Tumor-derived PGE2 was the main inducer of arginase I in regDCs and arginase I played the major role in suppressing CD4⁺ T cell response by regDCs [13].

Expression of arginase 1 in regDCs might cause dysregulation of the T cell receptor (TCR) signal and subsequently induces CD8⁺ T cell unresponsiveness [65]. T cell responses are profoundly suppressed by myeloid cell arginase-mediated L-arginine depletion and this has emerged as a fundamental mechanism of inflammation-associated immunosuppression [66]. T cell unresponsiveness due to L-arginine deficiency is mediated by down-

regulation of the TCR ζ chain and an arrest of T cells in the G0–G1 phase of the cell cycle, associated with the absence of up-regulation of cyclin D3 and cyclin-dependent kinase 4 (cdk4) [66]. It has been also reported that arginase 1 activation is associated with H_2O_2 production by MDSCs, which present class I-restricted epitopes directly to CD8⁺ T cells and inhibit their release of IFN- γ through the contact-dependent production of H_2O_2 [67]. However, the actual mechanism underlying the H_2O_2 generation following arginase 1 activation is not clear and may be linked to the synchronous activation of a different NOS isoform [68].

Additional Tolerogenic Pathways in regDCs: iNOS

Nitric oxide is a key molecule involved in the regulation of cell survival, proliferation and differentiation in many cell types. Several reports demonstrated that regulatory DC subsets strongly inhibited T cell response via NO production [12, 13]. NO can inhibit JAK3, which specifically binds to the common γ -chain that shared component of the IL-2, IL-4, IL-7, IL-9, and IL-15 receptors that control differentiation and proliferation of lymphocytes [69]. NO may also inhibit STAT5, which is critical to maintain effector CD8⁺ T cell responses and down-regulates the MHC class II expression required for T cell activation. NO-induced inhibition of mitogenic and peptide-specific T cell responses may not be associated with a loss in IL-2 production or inability to up-modulate the early activation markers, CD69 and CD25, but might result in direct impairment of the three IL-2R signaling pathways, as demonstrated by the lack of Janus kinase 3, STAT5, extracellular signal-regulated kinase, and Akt phosphorylation in response to IL-2 [70].

Additional Tolerogenic Pathways in regDCs: IDO

Suppression of T cell activation and proliferation by regDCs may be due to the expression of indoleamine-2,3-dioxygenase, a rate-limiting enzyme in the tryptophan catabolism, also known as the kynurenine pathway. IDO can be expressed by tumors themselves, but, in addition, its natural site of expression is the host immune cells recruited by the tumor, particularly DCs [71]. IDO has been implicated in tolerance and immunosuppression as it consumes the essential amino acid tryptophan, causing immune somnolence by promoting a semi-matured tolerogenic state on DCs, effector T cell anergy and expansion of Tregs at the site of IDO secretion [72–76]. IDO-expressing regDCs found at the tumor site and in tumor-draining lymph nodes [77–79] might help suppress the initiation of immune responses to tumor-derived antigens and perhaps help create systemic tolerance to these antigens [80, 81]. Therefore, expression of IDO in regDCs represents a key mechanism responsible for inducing the tolerogenic state.

Interestingly, bone marrow-derived DCs cultivated in the presence of vitamin D3 have been reported to present a tolerogenic profile with high IL-10, TNF- α , and IDO expression and decreased MHC-II and CD80 expression [82]. The adoptive transfer of IDO⁺regDCs in rodents induced a significant increase in the percentage of CD4⁺CD25⁺Foxp3⁺ T cells in the lymph nodes, comparable with the in vivo vitamin D3 treatment. Recently, prostaglandin E2 has been also reported to stimulate the IDO pathway in DCs [83].

Because of its potent immunosuppressive effects, IDO competence in DCs is tightly regulated, at the transcriptional, translational and post-translational levels [84]. However, the mechanisms by which IDO becomes dysregulated in the subset of tumor-associated regDCs have not yet been well characterized.

Additional Tolerogenic Pathways in regDCs: Neuropeptides

Although the effects of neuropeptides on DC generation, function and longevity have been repeatedly reported [85–87], including the effects of tumor cell-derived neuropeptides [88], the role of DC-derived neuropeptides in immune response regulation was not well determined. However, neuropeptides might be involved in functional activity of regDC in the inflammatory and probably tumor microenvironment. On contrary, several neuropeptides have been reported to induce the generation of immunosuppressive DCs. For instance, treatment of DCs with adrenomedullin, a neuropeptide with anti-apoptotic and anti-inflammatory effects, resulted in lower levels of co-stimulatory molecule expression and pro-inflammatory cytokine release. DCs express both adrenomedullin and its receptors and adrenomedullin stimulation induced IDO expression, promoting Treg cell expansion [89]. Interestingly, it has been recently reported that in melanomas, TAM-derived adrenomedullin is involved in angiogenesis and tumor growth. It was found that the adrenomedullin derived from TAMs interacts with its receptors on endothelial cells to promote tumor growth via a paracrine loop through the activation of the eNOS signaling pathway similar to the angiogenesis cytokine VEGF [90]. On the other hand, TAM-derived adrenomedullin can influence macrophages themselves in an autocrine manner, and, probably, may render immunosuppressive phenotype of tumor-associated DCs as well.

The neuropeptide α -melanocyte-stimulating hormone (α -MSH) is a well-known mediator of skin pigmentation. More recently, it has been shown that α -MSH also exerts a strong anti-inflammatory and immunosuppressive activity. Analysis of α -MSH effects on DCs revealed that α -MSH, by binding to MC-1R, induced regDCs, which were capable of expanding CD4⁺CD25⁺Foxp3⁺ regulatory T cells in

vitro, as well as in vivo [91]. Notably, α -MSH induced regDCs were capable of generating functional Tregs in human blood. Interestingly, human Tregs expanded via α -MSH-stimulated DCs suppressed the proliferation and cytokine secretion of T-helper-17 (Th17) cells [91]. As α -melanocyte-stimulating hormone is a ligand for the melanocortin 1 receptor (MC1R), which expressed on and play a role in malignant cell function through the Wnt/ β -catenin pathway [92], its role in formation and action of tumor-associated regDCs should be further investigated.

Additional Tolerogenic Pathways in regDCs: Other Mechanisms

Regulatory DC could express high level of Fas ligand (FasL) and induce apoptosis of activated CD4 T cells via FasL-mediated signaling. Stroma-derived TGF- β might be responsible for the induction of high-level FasL expression on regulatory DCs via ERK activation. Interestingly, FasL-enhanced IFN- γ from activated CD4 T cells could in turn induce high-level NO production from regulatory DCs, being involved in apoptosis induction of activated CD4 T cells [11]. In turn, DCs co-cultured with apoptotic cells up-regulate iNOS generation and STAT3 phosphorylation and can induce regulatory T cells after engulfing apoptotic cells. Importantly, DCs that phagocytose apoptotic T cells acquire inhibitory function toward CD4⁺ and CD8⁺ T cells [93]. These regDCs could not induce the generation of Tregs, but they were found to directly inhibit conventional DCs that initiate CD4⁺ and CD8⁺ T cell proliferation both in vitro and in vivo. Soluble factors including NO play a role in the DC-induced suppression of CD4⁺ and CD8⁺ T cell proliferation [93].

Other immunologically active molecules expressed by DCs may be also involved in active immunosuppression induced by regDCs. For instance, DCs constitutively express Galectin-1, -3, -8, and -9, a family of β -galactoside-binding proteins. Knockdown of Gal-1 or Gal-3 in DCs enhanced allogeneic T cell responses and IFN- γ but not IL-10 production [94], suggesting that DC-expressed Gal-1 and Gal-3 are regulatory molecules that favor the inhibition of T cell activation.

Furthermore, a dominant pathway of immune suppression in ovarian cancer involves regDC-associated B7-H1. The interaction of B7-H1 with PD-1 on tumor-infiltrating T cells is a widely cited theory of immune suppression involving B7-H1 in different cancers [95]. Recent studies suggest that the B7-H1 ligand, programmed death receptor-1 (PD-1), is also expressed on myeloid cells, complicating interpretations of how B7-H1 regulates DC function in the tumor. New data show that ovarian cancer-infiltrating DCs progressively expressed increased levels of PD-1 over time in addition to B7-H1 [96]. These dual-positive PD-1⁺B7-H1⁺ DCs have a classical DC phenotype, but are immature, suppressive, and respond poorly to danger signals.

Accumulation of PD-1⁺B7-H1⁺ DCs in the tumor was associated with suppression of T cell activity and decreased infiltrating T cells in advancing tumors. T cell suppressor function of these regDCs appeared to be mediated by T cell-associated PD-1 [96]. In contrast, ligation of PD-1 expressed on the tumor-associated DCs suppressed NF- κ B activation, release of immune regulatory cytokines, and upregulation of costimulatory molecules, revealing a novel role of tumor infiltrating PD-1⁺B7-H1⁺ regDCs in mediating immune suppression in ovarian cancer. Interestingly, in the lung cancer microenvironment, TGF- β up-regulates B7-H1 and GITRL expression in DCs, which was associated with regulatory T cell generation [97]. Analyzing expression of T cell coinhibitory B7 molecules (B7-DC, B7-1, B7-2, B7-H1, B7-H3) on DCs isolated from malignant and nonmalignant lung and lymph node tissue from patients with NSCLC, Schneider et al. reported that tumor induced an immunosuppressive phenotype of DCs by up-regulating B7-H3 [98].

Conclusion

Increasing evidence shows that the tumor microenvironment is immunosuppressive [99–101]. Tumor-induced immune suppression and unresponsiveness remain a constant obstacle to successful antitumor immune responses and, therefore, anti-cancer immunotherapy. As tumors develop and progress, they form a unique microenvironment that not only supports tumor growth, spreading and metastasis, but also diminish potential adaptive immunity to tumor antigens. Among the many components of this tumor microenvironment is a population of regDCs, which exert profound immune suppressive effects on T cells and, probably, other immune effector cells, and interact with different myeloid and lymphoid regulatory cells. Recently, different kinds of regulatory or tolerogenic DCs have been reported [1, 102]. Regulatory DCs can suppress T cell activation and proliferation via inducing Treg cell expansion or generation, or T cell anergy. Because tolerogenic or regulatory DCs can induce Treg cells, they are loosely designated as regDCs [19]. In addition to the induction of Treg cells, regDCs may also regulate directly the fate of effector CD4 T cells via other manners such as induction of T cell anergy. Several different mechanisms involving in DC-induced T cell inhibition have been described and their participation in the immunosuppressive properties of the tumor microenvironment is cancer-dependent. DC-mediated suppression of T cells may be due to IL-10, TGF- β , chemokines, neuropeptides, prostaglandins and other pathways. DC-derived macrophages, for instance, potently suppress T cell responses through the production of immunosuppressive molecules including nitric oxide, arginase, and IL-10 [103]. Understanding these mechanisms should provide

new means to harness tumor-mediated immunosuppression. For example, a variety of both biological and pharmacological agents can induce regDCs, and several in vitro studies have demonstrated that human regulatory T cells can be suppressed by DCs manipulated to decrease tolerogenic properties, with in vivo data also accumulating.

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