

Review Article

The Dendritic Cell-Regulatory T Lymphocyte Crosstalk Contributes to Tumor-Induced Tolerance

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Received 12 May 2011; Revised 30 August 2011; Accepted 31 August 2011

Academic Editor: Luigina Romani

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Tumor cells commonly escape from elimination by innate and adaptive immune responses using multiple strategies among which is the active suppression of effector immune cells. Regulatory T lymphocytes (Treg) and tolerogenic dendritic cells play essential roles in the establishment and persistence of cancer-induced immunosuppression. Differentiating dendritic cells (DCs) exposed to tumor-derived factors may be arrested at an immature stage becoming inept at initiating immune responses and may induce effector T-cell anergy or deletion. These tolerogenic DCs, which accumulate in patients with different types of cancers, are also involved in the generation of Treg. In turn, Treg that expand during tumor progression contribute to the immune tolerance of cancer by impeding DCs' ability to orchestrate immune responses and by directly inhibiting antitumoral T lymphocytes. Herein we review these bidirectional communications between DCs and Treg as they relate to the promotion of cancer-induced tolerance.

1. Introduction

Despite the arsenal harbored by the immune system to avert tumor development, cancers commonly elude immune detection and elimination by employing multiple strategies [1–5]. The past decade has witnessed considerable advances in our understanding of the mechanisms responsible for the resistance of tumor cells to immune control [6]. These include the downregulation or loss of expression by cancer cells of major histocompatibility complex (MHC) Class I molecules, resulting in the lack of recognition by cytotoxic T lymphocytes (CTL) [6–10]. Resistance to cell death (e.g., expression of antiapoptotic factors, deficiencies in the apoptosis cascade, deficiency in death receptor expression or function, blockade of perforin/granzyme) also contributes to avoidance of tumor cell killing by CTL [5, 11–15]. Additionally, cancer cells may produce immunosuppressive factors that negatively affect the function of DCs, T, and natural killer (NK) cells [11]. Nitric oxide (NO), IL-6, IL-10, tumor growth factor beta (TGF- β), indoleamine 2,3-dioxygenase (IDO), arginase-1, prostaglandin E2 (PGE₂), vascular

endothelial growth factor (VEGF), and cyclooxygenase-2 (COX-2) are examples of such molecules that can impede the proliferation and function of CD4⁺ and CD8⁺ T cells [5, 12, 16]. This immunosuppressive tumor environment may also foster the generation and/or promotion of immunosuppressive cells such as type 2 macrophages (M2), myeloid-derived suppressor cells (MDSCs), immature/tolerogenic DCs, and Treg [17–20].

By virtue of the immunosuppressive cytokines they secrete or through direct cell-cell contact interactions, both tolerogenic DCs and Treg can block antitumoral T- or NK cell activation and/or induce lymphocyte anergy or apoptosis [20–26]. Such properties place these cells at the center of tumor-induced immunosuppressive networks. Different mechanisms responsible for the accumulation of tolerogenic dendritic cells and Treg in cancer have been described but are still subjected to intensive investigation. One of them may involve a positive feedback loop by which tolerogenic DCs induce Treg that in turn contribute to the induction of immunocompromised DCs. We here review the bidirectional communications between tolerogenic DCs and Treg and

their roles in the context of tumor-induced immunosuppression.

2. The Central Role of Regulatory T Cells and Dendritic Cells in the Induction and Maintenance of an Immunosuppressive Tumor Microenvironment

2.1. Tolerogenic DCs and Their Contribution to Cancer-Induced Immunosuppression

2.1.1. DC Function Depends on Their Maturation and Activation Status. Known for years for their unique capability to function as professional antigen-presenting cells (APCs), DCs play a central role in the initiation and regulation of immune responses and are thereby essential for the protection against infectious pathogens and neoplastic cells [27–30]. DCs are endowed with the potential to activate antigen-specific effector T lymphocytes and are capable of promoting NKT and NK cell function [27, 31, 32]. The efficient stimulation of tumor-specific T lymphocytes by DCs requires the presentation of tumor-derived epitopes on MHC class I and II molecules together with second signals (costimulatory molecules CD80, CD86, CD40) and proinflammatory cytokines such as IL-12 or TNF- α [27, 31–33]. Immature DCs are characterized by high antigen uptake and processing capabilities, but by low expression of costimulatory molecules and thus are not capable of efficiently activating T cells. Multiple DC activation molecules including cytokines (such as interferons, TNF- α , GM-CSF, PGE₂, or IL-1 β), ligands of the TNF receptor family, or TLR ligands can act as “danger” signals when tissue damage occurs or pathogens are present [33–35]. These signals promote the differentiation of resident immature DCs into mature DCs characterized by the upregulation of MHC (class I and II) and costimulatory molecules (such as CD80/CD86, OX40L, ICOSL), the production of proinflammatory cytokines including IL-12, TNF- α , IL-1 β , or IL-6, and the ability to migrate, in response to specific chemokines, to the secondary lymphoid organs where they encounter naïve T cells [31, 36]. Only fully matured DCs are capable of priming and activating CD4⁺ and CD8⁺ T lymphocytes [34, 37, 38]. The ability of DCs to function as inducers of immunity thus depends on their activation/maturation stage.

Although traditionally viewed as the main inducers of immunity, DCs can also participate in the maintenance of peripheral self-tolerance [39, 40]. Under steady-state conditions, in the absence of inflammatory danger signals, immature DCs constantly engulf, process, and present self-antigens from apoptotic cells to potentially self-reactive T lymphocytes, resulting in T-cell anergy or deletion [40–42]. Migration of these immature DCs to the secondary lymphoid organs is contingent upon expression of CCR7, a chemokine receptor normally expressed by mature DCs. This mechanism is essential for the prevention of autoimmunity. In addition to anergizing antigen-specific T cells, these immature DCs have also been involved in the generation of Treg which further contributes to peripheral tolerance [43–46].

2.1.2. Immature/Tolerogenic DCs in Cancer. A profound deficit in the function of DCs (lack of costimulatory molecule expression, decreased production of proinflammatory cytokines, deficiency in the antigen processing and presenting machineries, inability of activating T lymphocytes) has been described in cancer-bearing hosts [26, 47–50]. In cancer patients, tumor-derived factors have been reported to alter DC differentiation and maturation and thereby promote the accumulation of immature DCs (iDCs) in the tumor (tumor-infiltrating DCs, TiDCs) and the lymph nodes. These immunocompromised DCs are unable to initiate anti-tumor immune responses but can tolerize T lymphocytes [20, 26, 39, 40, 51–54] and, as discussed in Section 3, contribute to the recruitment, expansion, and function of Treg [43, 46, 55–58]. For instance, TiDCs isolated from patients with breast cancer, ovarian cancer, head and neck or lung cancer express inhibitory molecules and fail to induce autologous T-cell proliferation [51, 59, 60]. In murine tumor models a subset of immature myeloid DCs is expanded in the tumor-draining lymph nodes. These immature DCs have decreased production of IL-12, TNF- α , and IL-6 and increased production of IL-10 and TGF- β and of IDO and are responsible for the establishment of an immunosuppressive environment [61]. Upregulation of immunosuppressive molecules such as B7-H4 also contributed to the tolerogenic characteristics of these DCs [62]. Immunocompromised DCs have also been found in rat cancer models. TiDCs expressing MHC class II and ICAM-1 but lacking costimulatory molecules are not capable of inducing allogeneic T-cell proliferation [63–65]. In addition to myeloid iDCs, accumulation of plasmacytoid DCs (pDCs) has also been found in the tumor-draining lymph nodes in B16 tumor-bearing mice [66] and in head and neck human tumors [67]. These pDCs are recruited to the tumor microenvironment in response to several chemokines, including CCL20, stromal cell-derived factor-1/CXCL12, and Ag-5/vascular cell adhesion molecule-1 interactions [68, 69]. The majority of these pDCs exhibit poor immunostimulatory capacity, express IDO, and may promote FoxP3⁺ Treg rather than activating effector T lymphocytes [70, 71]. In humans, the accumulation of IDO-expressing cells in melanoma [72–74], pancreatic ductal adenocarcinoma [75], ovarian cancer [76], colon cancer [77, 78], and non-small-cell lung cancer [79] has been associated with a worsened clinical outcome. However, in contrast to these observations, IDO expression in tumor endothelial cells of patients with renal cell carcinoma seems to restrict tumor growth and to contribute to long-term survival, possibly by limiting the influx of tryptophan from the blood to the tumor or by generating metabolites toxic to tumor cells [80]. These opposite results may be explained by the type of cells expressing IDO. In fact, unlike other malignancies where the main source of IDO is either the cancer cells themselves or tumor infiltrating leukocytes (DCs, eosinophils), in renal cell carcinoma IDO is almost exclusively expressed by endothelial cells of newly formed blood vessels. IDO expression by cells involved in the microvasculature has been associated with a Th-1-related cytokine milieu (mainly IFN- γ) [80] which may impair tumor growth. Consistently, high microvessel density correlates with lower tumor grade and prolonged

survival of patients with renal cell carcinoma [81]. Immature/tolerogenic DCs may also contribute to tumor development by fostering tumor angiogenesis. They are indeed capable of producing different cytokines and growth factors such as VEGF, promoting neoangiogenesis [82, 83].

Different approaches have been evaluated to correct the phenotypical and functional deficiencies of DCs in cancer, which include attempts to promote their maturation using different techniques. For example, the combination of CpG and anti-IL-10R antagonist has been reported to enhance IL-12 production and therefore the capacity of DCs to activate specific T cell *in vitro* and *in vivo* [84]. Interestingly, short-term ablation of DCs *in vivo* using a diphtheria toxin-based system has been reported to impair tumor growth in animal models [85].

Tumors have developed a series of strategies to suppress DC function. Some of the defined mechanisms underlying the blockade of DC maturation and the accumulation of tolerogenic DCs include the production of immunosuppressive factors such as TGF- β , IL-10, IL-6, VEGF, IDO, and PGE₂ [11, 18, 70, 86]. This results in the induction of inhibitory signaling pathways in DCs. One of them involves the transcription factor STAT-3, which plays a key role in the regulation of inflammatory processes [87]. Constitutive STAT-3 activation in tumors (both of hematopoietic and of epithelial origin) inhibits the production of proinflammatory cytokines by infiltrating immune cells while promoting the release of soluble factors that suppress DC function [87–92]. Furthermore, some tumor-derived molecules (VEGF, IL-6) enhance the expression of STAT-3 in DCs [20, 91, 92]. STAT-3 activation, although an important event in early differentiation of DCs, is decreased in fully differentiated mature DCs [91]. Tumor-induced maintenance of constitutive STAT-3 activation in DCs eventually results in the acquisition of the tolerogenic potential of these cells [91, 93–98]. Expectedly, the disruption of STAT-3 signaling, for example, using dominant negative STAT-3 variants in the mouse, leads to tumor regression or growth control *in vivo* [90, 98, 99]. Similarly, the cytokine signaling inhibitor SOCS-1 has been highlighted as an important regulator of DC APC function [100]. The inhibition of this molecule using specific siRNA has been reported to break tolerance to the self-antigen Trp2 in an established B16 tumor model [100].

In addition to the mechanisms described above, tumor-induced Treg may also participate in the inhibition of DC maturation and thus in the generation of tolerogenic DCs.

2.2. Regulatory T Cells Critically Contribute to Tumor-Induced Tolerance

2.2.1. Regulatory T Lymphocytes.

Initially described in the field of autoimmunity, regulatory T cells (Treg) are comprised of a heterogeneous population of T lymphocytes defined by their capacity to suppress immune responses to self- and foreign antigens [23, 101–105]. Treg can act as critical checkpoints in the control of autoimmunity, infections, or cancer [19, 23, 101, 106–110]. A wide diversity of immunosuppressive T cells have been identified [101]. As a member

of the growing family of immunosuppressive/regulatory T lymphocytes [23, 101, 107], the CD4⁺CD25⁺ Treg subset has been extensively studied over the last two decades. These cells constitute about 10% of the circulating T-lymphocyte population in mice and 5% in healthy humans [111]. In addition to CD25, the α -chain of the IL-2 receptor, this lymphocyte subpopulation also expresses multiple markers including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), glucocorticoid-induced TNF receptor (GITR), CD62L, lymphocyte activation gene 3 (LAG 3), Toll-like receptors (TLR-4, -5, -7, -8) [112]. In human, the IL-7 receptor (CD127) has been used to distinguish Treg from activated T cells. CD127 expression has indeed been reported to inversely correlate with FoxP3 expression and the suppressive function of Treg [113, 114]. However, increased CD127 expression has also been detected on activated (ICOS- and CD103-expressing) Treg subsets [115]. Expression of the ectonucleotidase CD39 by FoxP3⁺ Treg has been reported in mouse and human [116]. However, in contrast to mice, in human this enzyme seems to be restricted to a subset of FoxP3⁺ regulatory effector/memory-like T (Trem) cells [116]. CD39 together with another ectoenzyme (CD73) is involved in the generation of pericellular adenosine from extracellular nucleotides, resulting in the suppression of adenosine A2A receptor-expressing activated T-effector cells [117]. The forkhead/winged helix transcription factor FoxP3 appears fundamental for the development and function of CD4⁺CD25⁺ Treg and remains the most specific molecular marker for these cells [112, 118–121]. Treg contribute to the prevention of autoimmune diseases by controlling the activity of autoreactive T lymphocytes that have escaped negative selection in the thymus [103, 105, 122]. Elimination of Treg or genetic alteration of the FoxP3 gene results in the development of lethal autoimmune conditions, evidencing the essential role of these cells in the maintenance of active dominant peripheral tolerance [111, 123–125]. Depending on their origin, two types of CD4⁺CD25⁺FoxP3⁺ Treg can be identified. Naturally occurring Treg (natural or nTreg) that develop in the thymus and adaptive (inducible or iTreg) are generated by the conversion of CD4⁺CD25⁻ naïve T cells in the periphery [126–128]. It has been documented that Treg survival and immunosuppressive function and Treg production from naïve T cells depend on external signals, some of which are relayed by the TCR, CD28, TGF- β , and IL-2 receptors and other yet to be identified molecules [101–103, 129–132], converging towards the regulation of specific gene expression such as FoxP3. Although most iTreg are characterized by a CD25^{high} phenotype, the generation of CD25⁻ Treg by coimmunization with highly antigenic epitopes has also been reported [133]. In addition, the significance of CD25 expression by Treg is subjected to discussion, and T cells with regulatory properties have also been detected in the CD4⁺CD25⁻ subset [134–136]. The cellular and molecular bases for the suppressive activity of CD4⁺CD25⁺ Treg cells remain contentious [101, 119, 137–140]. Some proposed mechanisms include the production of inhibitory cytokines such as IL-10, TGF- β , and IL-35, a direct cell contact involving CTLA-4 and CD80/CD86, expression of granzymes, the depletion of IL-2 from the environment,

the transfer of cAMP to the target cells, the release of nucleosides, and other yet unidentified mechanisms [23, 138, 141–148].

2.2.2. Role of Treg in Cancer. Multiple studies have demonstrated that, besides their role in autoimmunity, Treg critically contribute to the immune tolerance of cancer. An increase in the number of these cells has been detected in the blood, lymph nodes, and spleen of tumor-bearing hosts and correlates with poor prognosis [24, 48, 127, 149–153]. Treg expansion observed during tumor progression may result from the proliferation of nTreg or from the conversion of CD4⁺CD25⁻FoxP3⁻ T cells into CD4⁺CD25⁺FoxP3⁺ iTreg [19, 126]. These two mechanisms may be complementary and may act in concert to achieve an optimal Treg expansion as reviewed in [102, 103, 111, 154]. In addition, it has been documented that a variety of tumors including breast cancer, melanoma, and lymphoma may recruit Treg to the tumor site. This Treg recruitment may involve a CCR4-dependent trafficking induced by CCL22 released by tumor cells and immune cells infiltrating the tumors such as macrophages and DCs [155]. This attraction of Treg by cancer cells and the modulation of Treg trafficking by tumor may be an essential element for the accumulation of Treg in the tumor microenvironment and for the mode of action of these cells in cancer [19, 106, 120, 127, 151, 156–159]. Treg impede antitumoral immune responses by suppressing the function of effectors CD4⁺, CD8⁺, and NK cells [24, 160–164] and also by inhibiting DC activation [48, 144, 165–168] as discussed in Section 4.

Since Treg represent a major obstacle for the elimination of tumors by immune cells, their therapeutic depletion or their functional inactivation using drugs or antibodies has been shown to improve responses to cancer immunotherapy including DC-based vaccines [150, 163, 169–171]. Different strategies have thus been explored to deplete/inactivate Treg *in vivo* [150, 163, 169–186]. However, the selective elimination or inactivation of Treg still constitutes a major challenge in immunotherapy since these cells share the same surface markers as activated conventional nonsuppressive T cells. Antibody-based approaches indistinctly target both Treg and activated effector T lymphocytes, and in most cases chemotherapeutic agents used to eliminate Treg do not exert specific effects on these cells. We have shown in the rat that cyclophosphamide administration results in elimination of both regulatory and effector T cells but that effector cell reconstitution occurs earlier than that of Treg [150]. Cyclophosphamide therapy enhanced tumor-specific vaccination [150]. At a low dose cyclophosphamide has been shown to trigger apoptosis of mouse Tregs *in vitro* and *in vivo* without significant changes in CD4⁺CD25⁻ cell viability [183, 187, 188]. However, clinical studies have also indicated that cyclophosphamide may not significantly affect Treg number and function [189]. Elimination of Treg based on CD25 expression results in the concurrent depletion of activated effector lymphocytes [154]. In addition, this strategy may foster tumor-driven conversion of Treg from CD4⁺CD25⁻FoxP3⁻ T cells [154, 185].

3. Promotion of Treg Expansion and Function by DCs

The mechanisms controlling the induction and maintenance of Treg during tumor development are still being elucidated. As outlined above, although critical for the development of adaptive immune responses, DCs may also contribute to the mechanisms of immune tolerance. These “tolerogenic” DCs of both plasmacytoid (pDCs) or myeloid (mDCs) origin are not only capable of anergizing effector T lymphocytes but may also be endowed with the capacity to drive the differentiation and/or proliferation of FoxP3⁺ Treg [39, 43, 46, 53, 58, 67, 190–199]. The ability of DCs to induce immune tolerance is believed to depend on their origin, activation state, the nature of the maturation signals and the cytokine context at the time they encounter T lymphocytes. Different subsets of tolerogenic DCs capable of promoting Treg expansion and/or function have been described [53, 57, 192, 195, 199, 200]. In physiological conditions, steady-state immature myeloid DCs constantly engulf and process self-antigens and upon migration to the draining lymph nodes can block self-reactive effector T cells and promote Treg expansion [39, 40, 58], thus contributing to the prevention of autoimmunity. In addition, semimature myeloid DCs, which exhibit some of the characteristics of mature DCs (including costimulatory molecule expression) but that produce significantly lower level of proinflammatory cytokines, have also been described for their ability to drive the differentiation of adaptive Treg [20, 39, 55, 196, 201, 202]. Importantly, phenotypically mature DCs not only induce immunity but may also exhibit a tolerogenic function. For instance, DCs isolated from Peyer’s patches, lungs, or the anterior chamber of the eye display a mature phenotype, secrete IL-10, and are capable of inducing Treg [200]. CD40L-activated pDCs may also be tolerogenic and support Treg expansion [43, 203]. In addition, following extensive stimulation *in vitro* with maturation signals (e.g., LPS), DCs become “exhausted” and produce IL-10 but not IL-12 and elicit nonpolarized memory cells and/or Th2 responses [204]. Whether these “exhausted” DCs may also induce Treg *in vivo* remains however to be determined. In addition, variable results have been reported as to whether mature or immature DCs may preferentially lead to Treg induction [55, 200].

The mechanisms underlying DC-mediated induction of Treg are still not entirely clear. Evidence has been provided that IDO, a key-enzyme that catalyses the degradation of the essential amino acid tryptophan into kynurenine, may play an important role in this process [70, 205]. IDO-mediated tryptophan deprivation from the T-lymphocyte environment results in the downregulation of TCR- ζ -chain and leads to the activation of the GCN2 (general control nonrepressed 2) kinase pathway that prevents T-cell cycling and activation [206, 207]. In addition the byproducts of the tryptophan catabolism such as L-kynurenine, 3-hydroxykynurenine, or 3-hydroxyanthranilic acid may be endowed with inherent suppressive activity [206, 207]. IDO can be expressed by different DC subsets in mouse and human [208]. Although CD8⁺ DCs and plasmacytoid DCs were originally identified as the main source of IDO, it has recently been shown that CD8a⁻ IDO⁻

DCs can be converted into IDO⁺ tolerogenic DCs [209]. IDO expression has been identified as a possible factor involved in DC-mediated induction of Treg [66]. In mice and human it has been reported that IDO⁺ DCs are able to promote the differentiation of iTreg from a pool of naïve T cells [206–208, 210]. Treg induction and activation by IDO⁺ DCs require the GCN2 pathway and may be prevented by CTLA-4 blockade [66]. It has also been shown that the production of TGF- β by DCs conditioned by the tumor microenvironment also promotes iTreg generation [126]. TGF- β , together with TCR and CD28 ligation, induces an intracellular signaling that involves the cytosolic Smad proteins (Smad 2 and 3) and STAT-3 and -5 activation, resulting in FoxP3 expression [112, 118, 126, 211]. Engagement of T-cell CTLA-4 and GITR by their ligands on DCs induces the activation of preexisting Treg as well as their *de novo* generation [66, 156, 208, 210]. The engagement of programmed death receptor-1 (PD-1) expressed by T cells with B7-H1 expressed by DCs and macrophages results in the negative regulation of target T lymphocytes [212]. B7-H1-expressing DCs generated in the tumor environment exhibit reduced T-cell stimulatory capacity and have been reported to foster Treg expansion by conversion of naïve T cells into iTreg and/or by promoting the proliferation of nTreg [212–215].

The homing of Treg to the tumor site or to the tumor-draining lymph nodes where they interact with their targets is essential for their role in cancer-induced tolerance. DCs are capable of modulating the trafficking and therefore the recruitment of Treg to the tumor site or to the secondary lymphoid organs [44, 155, 216]. Blood Treg have been shown to express high CCR4 and to selectively migrate in response to the CCR4 ligand CCL22 produced by tumor cells but also by tumor infiltrating DCs [127, 217–221].

In summary, DCs subverted by the tumor microenvironment lack effector T-cell stimulatory capacity but are endowed with the ability to promote suppressive Treg. In addition to tumor-derived factors which can directly induce Treg proliferation and/or generation from naïve T cells, DCs that differentiate in the tumor microenvironment provide essential signals that contribute to Treg expansion. Induction of Treg by DCs thus appears as one essential mechanism employed by cancers to generate immunosuppressive Treg and thereby to escape from antitumor immune responses (Figure 1).

4. Treg Negatively Modulate DC Maturation and Promote the Generation of Tolerogenic DCs

These interactions between immunosuppressive/tolerogenic DCs and Treg are not unidirectional, and Treg can “talk back” to DCs, influencing their maturation status (Figure 1). In a nontumor setting, the downregulation of DC costimulatory molecule expression [144] and IL-12 secretion [167] by Treg has been documented in the mouse. Human Treg have also been reported to exhibit suppressive effects on monocyte/macrophages [168] and on DCs generated from peripheral blood monocytes [166]. An inhibition by Treg of the maturation induced by a cocktail of TLR ligands of

human myeloid but not plasmacytoid DCs has also been reported [222]. Other studies have indicated that Treg may suppress DC costimulatory molecules CD80 and CD86 without affecting CD40 expression and that inhibition of DC maturation occurs in the absence of CD40-CD40L interaction [198]. In tumor immunity, Treg have primarily been described for their ability to impair the function of tumor-specific CD4⁺ and CD8⁺ T cells [102, 106, 223]. However, it has been reported that Treg from tumor-bearing mice may impair the expression of DC costimulatory molecules CD80, CD86, and CD40, suppress DC production of proinflammatory cytokines IL-12 and TNF- α , and inhibit their ability to induce T-cell activation *in vitro* [48, 165]. A proposed mechanism underlying tumor-induced Treg-mediated suppression of DCs may involve the suppressive cytokines TGF- β and IL-10 [48].

Treg have also been reported to induce the expression of the immunosuppressive molecules B7-H3 and B7-H4 on DCs [44, 224–226]. B7-H3 and B7-H4 are members of the B7 family, but, in contrast to their activating counterparts, they trigger inhibitory signals in T lymphocytes and thus contribute to the immunosuppressive function of DCs and thereby to cancer-induced tolerance [44, 212, 225]. These modifications in the expression of DC surface markers may depend on diverse mechanisms, and, in addition to CTLA-4, a role for LFA-1 (lymphocyte function-associated antigen 1), LAG-3 [227], and neuropilin-1 has been proposed [227]. The engagement of the B7 molecules on DCs by CTLA-4 on Treg has been shown to upregulate IDO production in human and murine DCs which then promote Treg [206]. In turn, IDO-activated Treg have been shown to induce PD-L1 upregulation on DCs [66, 207] resulting in an efficient feedback amplification loop [66]. An additional mechanism by which Treg may promote tolerogenic DCs involves the induction of IL-10 production by DC [226].

Importantly, mature DCs have been shown to be refractory to Treg-mediated inhibition and seem to display a stable phenotype when exposed to these suppressive cells [144, 222]. Mouse bone-marrow-derived DCs first activated with the TLR4 ligand LPS and exposed to tumor-induced Treg maintain expression of CD80, CD86, and CD40, produce IL-12 or TNF- α , and are not impaired in their allostimulatory activity [48]. This resistance of mature DCs to Treg suppression has therapeutic implications as it underlines the importance of activating *in vitro* DCs used as vaccines prior to their administration.

Thus, Treg contribute to tumor-induced tolerance by restraining DC maturation, proinflammatory cytokine production, and APC function, therefore participating in the induction and accumulation of tolerogenic DCs.

5. Conclusion

There is clear evidence that DCs rendered tolerogenic by the immunosuppressive tumor microenvironment are capable not only of inhibiting effector antitumoral T cells but also of promoting the differentiation of iTreg from naïve T lymphocytes or of fostering the proliferation of nTreg. Reciprocally,

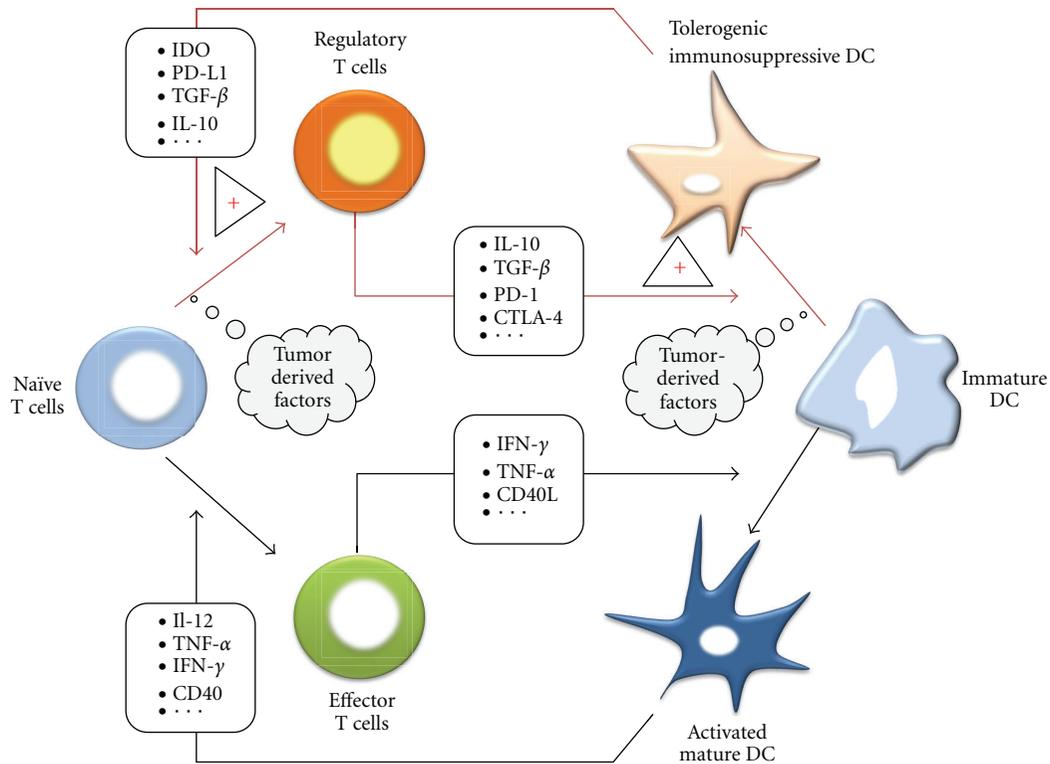


FIGURE 1: Bidirectional communications between Treg and tolerogenic DCs in cancer. Tumor-derived factors can promote the differentiation of immature DCs and naïve T cells into tolerogenic DCs and Treg. Tolerogenic DCs contribute to the generation of Treg by various mechanisms. In turn, Treg participate in tumor-induced tolerance by restraining DC maturation and fostering the accumulation of tolerogenic DCs.

cancer-induced Treg, by restraining DC maturation and by inducing DC expression and production of immunosuppressive molecules, may skew their differentiation towards a tolerogenic cell population. This positive feedback loop by which suppressed/tolerogenic DCs may induce Treg that in turn enhance DC immune inhibitory function may significantly contribute to the persistence of the immune tolerance to cancer.

These DC-Treg interactions, by enhancing tumor-induced immunosuppression, represent a major barrier to successful immunotherapy. Therefore, targeting the generation of these two suppressive cell populations is a desirable goal in chemo- and immunotherapeutic approaches. To achieve this objective there is a need to further improve strategies to simultaneously promote the full activation of DC using selective adjuvants such as TLR ligands or cytokines and impair Treg expansion, function, and recruitment.

Acknowledgments

The authors thank Collin J. LaCasse for his comments. Grant support: NIH Grant R01 CA104926 (E. Katsanis and N. Larmonier), AZ Cancer Center Support Grant CA023074 (E. Katsanis and N. Larmonier), and Tee Up for Tots and PANDA Foundations (E. Katsanis and N. Larmonier).

References

- [1] K. Staveley-O'Carroll, E. Sotomayor, J. Montgomery et al., "Induction of antigen-specific T cell anergy: an early event in the course of tumor progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 3, pp. 1178–1183, 1998.
- [2] M. J. Smyth, D. I. Godfrey, and J. A. Trapani, "A fresh look at tumor immunosurveillance and immunotherapy," *Nature Immunology*, vol. 2, no. 4, pp. 293–299, 2001.
- [3] M. B. Feinberg and G. Silvestri, "T(S) cells and immune tolerance induction: a regulatory renaissance?" *Nature Immunology*, vol. 3, no. 3, pp. 215–217, 2002.
- [4] D. Pardoll, "Does the immune system see tumors as foreign or self?" *Annual Review of Immunology*, vol. 21, pp. 807–839, 2003.
- [5] L. Zitvogel, A. Tesniere, and G. Kroemer, "Cancer despite immunosurveillance: immunoselection and immunosubversion," *Nature Reviews Immunology*, vol. 6, no. 10, pp. 715–727, 2006.
- [6] H. T. Khong and N. P. Restifo, "Natural selection of tumor variants in the generation of "tumor escape" phenotypes," *Nature Immunology*, vol. 3, no. 11, pp. 999–1005, 2002.
- [7] N. P. Restifo, F. Esquivel, Y. Kawakami et al., "Identification of human cancers deficient in antigen processing," *Journal of Experimental Medicine*, vol. 177, no. 2, pp. 265–272, 1993.
- [8] D. J. Hicklin, Z. Wang, F. Arienti, L. Rivoltini, G. Parmiani, and S. Ferrone, " β 2-Microglobulin mutations, HLA class I

- antigen loss, and tumor progression in melanoma," *Journal of Clinical Investigation*, vol. 101, no. 12, pp. 2720–2729, 1998.
- [9] N. P. Restifo, F. M. Marincola, Y. Kawakami, J. Taubenberger, J. R. Yannelli, and S. A. Rosenberg, "Loss of functional beta2-microglobulin in metastatic melanomas from five patients receiving immunotherapy," *Journal of the National Cancer Institute*, vol. 88, no. 2, pp. 100–108, 1996.
- [10] F. Garrido, F. Ruiz-Cabello, T. Cabrera et al., "Implications for immunosurveillance of altered HLA class I phenotypes in human tumours," *Immunology Today*, vol. 18, no. 2, pp. 89–95, 1997.
- [11] G. A. Rabinovich, D. Gabrilovich, and E. M. Sotomayor, "Immunosuppressive strategies that are mediated by tumor cells," *Annual Review of Immunology*, vol. 25, pp. 267–296, 2007.
- [12] B. Lu and O. J. Finn, "T-cell death and cancer immune tolerance," *Cell Death and Differentiation*, vol. 15, no. 1, pp. 70–79, 2008.
- [13] D. Vucic and W. J. Fairbrother, "The inhibitor of apoptosis proteins as therapeutic targets in cancer," *Clinical Cancer Research*, vol. 13, no. 20, pp. 5995–6000, 2007.
- [14] N. Rampino, H. Yamamoto, Y. Ionov et al., "Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype," *Science*, vol. 275, no. 5302, pp. 967–969, 1997.
- [15] J. P. Medema, J. de Jong, L. T. C. Peltenburg et al., "Blockade of the granzyme B/perforin pathway through overexpression of the serine protease inhibitor PI-9/SPI-6 constitutes a mechanism for immune escape by tumors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 20, pp. 11515–11520, 2001.
- [16] A. P. Vicari, C. Caux, and G. Trinchieri, "Tumour escape from immune surveillance through dendritic cell inactivation," *Seminars in Cancer Biology*, vol. 12, no. 1, pp. 33–42, 2002.
- [17] S. Nagaraj and D. I. Gabrilovich, "Tumor escape mechanism governed by myeloid-derived suppressor cells," *Cancer Research*, vol. 68, no. 8, pp. 2561–2563, 2008.
- [18] D. Gabrilovich, "Mechanisms and functional significance of tumour-induced dendritic-cell defects," *Nature Reviews Immunology*, vol. 4, no. 12, pp. 941–952, 2004.
- [19] W. Zou, "Regulatory T cells, tumour immunity and immunotherapy," *Nature Reviews Immunology*, vol. 6, no. 4, pp. 295–307, 2006.
- [20] I. Fricke and D. I. Gabrilovich, "Dendritic cells and tumor microenvironment: a dangerous liaison," *Immunological Investigations*, vol. 35, no. 3–4, pp. 459–483, 2006.
- [21] S. Kusmartsev and D. I. Gabrilovich, "Role of immature myeloid cells in mechanisms of immune evasion in cancer," *Cancer Immunology, Immunotherapy*, vol. 55, no. 3, pp. 237–245, 2006.
- [22] M. Terme, N. Chaput, B. Combadiere, M. Averil, T. Ohteki, and L. Zitvogel, "Regulatory T cells control dendritic cell/NK cell cross-talk in lymph nodes at the steady state by inhibiting CD4+ self-reactive T cells," *Journal of Immunology*, vol. 180, no. 7, pp. 4679–4686, 2008.
- [23] E. M. Shevach, "Mechanisms of Foxp3+ T regulatory cell-mediated suppression," *Immunity*, vol. 30, no. 5, pp. 636–645, 2009.
- [24] P. A. Antony, C. A. Piccirillo, A. Akpınarlı et al., "CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells," *Journal of Immunology*, vol. 174, no. 5, pp. 2591–2601, 2005.
- [25] C. A. Piccirillo and E. M. Shevach, "Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells," *Journal of Immunology*, vol. 167, no. 3, pp. 1137–1140, 2001.
- [26] B. Almand, J. I. Clark, E. Nikitina et al., "Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer," *Journal of Immunology*, vol. 166, no. 1, pp. 678–689, 2001.
- [27] J. Banchereau and R. M. Steinman, "Dendritic cells and the control of immunity," *Nature*, vol. 392, no. 6673, pp. 245–252, 1998.
- [28] G. J. Adema, "Dendritic cells from bench to bedside and back," *Immunology Letters*, vol. 122, no. 2, pp. 128–130, 2009.
- [29] R. M. Steinman and J. Banchereau, "Taking dendritic cells into medicine," *Nature*, vol. 449, no. 7161, pp. 419–426, 2007.
- [30] R. M. Steinman, "Some interfaces of dendritic cell biology," *APMIS*, vol. 111, no. 7–8, pp. 675–697, 2003.
- [31] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.
- [32] A. K. Palucka, H. Ueno, J. Fay, and J. Banchereau, "Dendritic cells: a critical player in cancer therapy?" *Journal of Immunotherapy*, vol. 31, no. 9, pp. 793–805, 2008.
- [33] N. Janikashvili, N. Larmonier, and E. Katsanis, "Personalized dendritic cell-based tumor immunotherapy," *Immunotherapy*, vol. 2, no. 1, pp. 57–68, 2010.
- [34] J. Fraszczak, M. Trad, N. Janikashvili et al., "Peroxy-nitrite-dependent killing of cancer cells and presentation of released tumor antigens by activated dendritic cells," *Journal of Immunology*, vol. 184, no. 4, pp. 1876–1884, 2010.
- [35] N. Larmonier, J. Fraszczak, D. Lakomy, B. Bonnotte, and E. Katsanis, "Killer dendritic cells and their potential for cancer immunotherapy," *Cancer Immunology, Immunotherapy*, vol. 59, no. 1, pp. 1–11, 2010.
- [36] C. J. M. Melief, "Regulation of cytotoxic T lymphocyte responses by dendritic cells: peaceful coexistence of cross-priming and direct priming?" *European Journal of Immunology*, vol. 33, no. 10, pp. 2645–2654, 2003.
- [37] A. Lin, A. Schildknecht, L. T. Nguyen, and P. S. Ohashi, "Dendritic cells integrate signals from the tumor microenvironment to modulate immunity and tumor growth," *Immunology Letters*, vol. 127, no. 2, pp. 77–84, 2010.
- [38] H. Conroy, N. A. Marshall, and K. H. G. Mills, "TLR ligand suppression or enhancement of Treg cells? A double-edged sword in immunity to tumours," *Oncogene*, vol. 27, no. 2, pp. 168–180, 2008.
- [39] R. M. Steinman, D. Hawiger, and M. C. Nussenzweig, "Tolerogenic dendritic cells," *Annual Review of Immunology*, vol. 21, pp. 685–711, 2003.
- [40] R. M. Steinman, D. Hawiger, K. Liu et al., "Dendritic cell function in vivo during the steady state: a role in peripheral tolerance," *Annals of the New York Academy of Sciences*, vol. 987, pp. 15–25, 2003.
- [41] R. Kushwah, J. Wu, J. R. Oliver et al., "Uptake of apoptotic DC converts immature DC into tolerogenic DC that induce differentiation of Foxp3+ Treg," *European Journal of Immunology*, vol. 40, no. 4, pp. 1022–1035, 2010.
- [42] S. Rutella, S. Danese, and G. Leone, "Tolerogenic dendritic cells: cytokine modulation comes of age," *Blood*, vol. 108, no. 5, pp. 1435–1440, 2006.
- [43] P. Hubert, N. Jacobs, J. H. Caberg, J. Boniver, and P. Delvenne, "The cross-talk between dendritic and regulatory T

- cells: good or evil?" *Journal of Leukocyte Biology*, vol. 82, no. 4, pp. 781–794, 2007.
- [44] K. Mahnke, T. Bedke, and A. H. Enk, "Regulatory conversation between antigen presenting cells and regulatory T cells enhance immune suppression," *Cellular Immunology*, vol. 250, no. 1-2, pp. 1–13, 2007.
- [45] K. Mahnke, J. Knop, and A. H. Enk, "Induction of tolerogenic DCs: 'You are what you eat,'" *Trends in Immunology*, vol. 24, no. 12, pp. 646–651, 2003.
- [46] H. Jonuleit, E. Schmitt, G. Schuler, J. Knop, and A. H. Enk, "Induction of interleukin 10-producing, nonproliferating CD4+ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells," *Journal of Experimental Medicine*, vol. 192, no. 9, pp. 1213–1222, 2000.
- [47] S. Kusmartsev and D. I. Gabrilovich, "Effect of tumor-derived cytokines and growth factors on differentiation and immune suppressive features of myeloid cells in cancer," *Cancer and Metastasis Reviews*, vol. 25, no. 3, pp. 323–331, 2006.
- [48] N. Larmonier, M. Marron, Y. Zeng et al., "Tumor-derived CD4+CD25+ regulatory T cell suppression of dendritic cell function involves TGF- β and IL-10," *Cancer Immunology, Immunotherapy*, vol. 56, no. 1, pp. 48–59, 2007.
- [49] P. Chaux, N. Favre, B. Bonnotte, M. Moutet, M. Martin, and F. Martin, "Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression—a role in the immune tolerance to antigenic tumors," *Advances in Experimental Medicine and Biology*, vol. 417, pp. 525–528, 1997.
- [50] P. Monti, B. E. Leone, A. Zerbi et al., "Tumor-derived MUC1 mucins interact with differentiating monocytes and induce IL-10highIL-12low regulatory dendritic cell," *Journal of Immunology*, vol. 172, no. 12, pp. 7341–7349, 2004.
- [51] L. A. Norian, P. C. Rodriguez, L. A. O'Mara et al., "Tumor-Infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-Arginine metabolism," *Cancer Research*, vol. 69, no. 7, pp. 3086–3094, 2009.
- [52] D. I. Gabrilovich, H. L. Chen, K. R. Girgis et al., "Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells," *Nature Medicine*, vol. 2, no. 10, pp. 1096–1103, 1996.
- [53] D. Hawiger, K. Inaba, Y. Dorsett et al., "Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo," *Journal of Experimental Medicine*, vol. 194, no. 6, pp. 769–779, 2001.
- [54] C. Melani, C. Chiodoni, G. Forni, and M. P. Colombo, "Myeloid cell expansion elicited by the progression of spontaneous mammary carcinomas in c-erbB-2 transgenic BALB/c mice suppresses immune reactivity," *Blood*, vol. 102, no. 6, pp. 2138–2145, 2003.
- [55] R. A. Maldonado and U. H. von Andrian, "How tolerogenic dendritic cells induce regulatory T cells," *Advances in Immunology*, vol. 108, pp. 111–165, 2010.
- [56] S. Yamazaki and R. M. Steinman, "Dendritic cells as controllers of antigen-specific Foxp3+ regulatory T cells," *Journal of Dermatological Science*, vol. 54, no. 2, pp. 69–75, 2009.
- [57] S. Yamazaki, K. Inaba, K. V. Tarbell, and R. M. Steinman, "Dendritic cells expand antigen-specific Foxp3+CD25+CD4+ regulatory T cells including suppressors of alloreactivity," *Immunological Reviews*, vol. 212, pp. 314–329, 2006.
- [58] K. Mahnke, Y. Qian, J. Knop, and A. H. Enk, "Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells," *Blood*, vol. 101, no. 12, pp. 4862–4869, 2003.
- [59] M. Zhang, H. Tang, Z. Guo et al., "Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells," *Nature Immunology*, vol. 5, no. 11, pp. 1124–1133, 2004.
- [60] C. Aspod, A. Pedroza-Gonzalez, M. Gallegos et al., "Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development," *Journal of Experimental Medicine*, vol. 204, no. 5, pp. 1037–1047, 2007.
- [61] P. Stoitzner, L. K. Green, J. Y. Jung et al., "Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells," *Cancer Immunology, Immunotherapy*, vol. 57, no. 11, pp. 1665–1673, 2008.
- [62] C. Cheng, Q. X. Qu, Y. Shen et al., "Overexpression of B7-H4 in tumor infiltrated dendritic cells," *Journal of Immunoassay and Immunochemistry*, vol. 32, no. 4, pp. 353–364, 2011.
- [63] P. Chaux, N. Favre, M. Martin, and F. Martin, "Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression in rats," *International Journal of Cancer*, vol. 72, no. 4, pp. 619–624, 1997.
- [64] B. Bonnotte, M. Crittenden, N. Larmonier, M. Gough, and R. G. Vile, "MIP-3 α transfection into a rodent tumor cell line increases intratumoral dendritic cell infiltration but enhances (facilitates) tumor growth and decreases immunogenicity," *Journal of Immunology*, vol. 173, no. 8, pp. 4929–4935, 2004.
- [65] P. Chaux, M. Moutet, J. Faivre, F. Martin, and M. Martin, "Inflammatory cells infiltrating human colorectal carcinomas express HLA class II but not B7-1 and B7-2 costimulatory molecules of the T-cell activation," *Laboratory Investigation*, vol. 74, no. 5, pp. 975–983, 1996.
- [66] M. D. Sharma, B. Baban, P. Chandler et al., "Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase," *Journal of Clinical Investigation*, vol. 117, no. 9, pp. 2570–2582, 2007.
- [67] E. Hartmann, B. Wollenberg, S. Rothenfusser et al., "Identification and functional analysis of tumor-infiltrating plasmacytoid dendritic cells in head and neck cancer," *Cancer Research*, vol. 63, no. 19, pp. 6478–6487, 2003.
- [68] W. Zoul, V. Machelon, A. Coulomb-L'Hermin et al., "Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells," *Nature Medicine*, vol. 7, no. 12, pp. 1339–1346, 2001.
- [69] J. Charles, J. di Domizio, D. Salameire et al., "Characterization of circulating dendritic cells in melanoma: role of CCR6 in plasmacytoid dendritic cell recruitment to the tumor," *Journal of Investigative Dermatology*, vol. 130, no. 6, pp. 1646–1656, 2010.
- [70] D. H. Munn and A. L. Mellor, "Indoleamine 2,3-dioxygenase and tumor-induced tolerance," *Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1147–1154, 2007.
- [71] D. H. Munn, M. D. Sharma, D. Hou et al., "Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes," *Journal of Clinical Investigation*, vol. 114, no. 2, pp. 280–290, 2004.
- [72] G. Weinlich, C. Murr, L. Richardsen, C. Winkler, and D. Fuchs, "Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients," *Dermatology*, vol. 214, no. 1, pp. 8–14, 2007.

- [73] J. R. Brody, C. L. Costantino, A. C. Berger et al., "Expression of indoleamine 2,3-dioxygenase in metastatic malignant melanoma recruits regulatory T cells to avoid immune detection and affects survival," *Cell Cycle*, vol. 8, no. 12, pp. 1930–1934, 2009.
- [74] J. R. Lee, R. R. Dalton, J. L. Messina et al., "Pattern of recruitment of immunoregulatory antigen-presenting cells in malignant melanoma," *Laboratory Investigation*, vol. 83, no. 10, pp. 1457–1466, 2003.
- [75] A. Witkiewicz, T. K. Williams, J. Cozzitorto et al., "Expression of indoleamine 2,3-dioxygenase in metastatic pancreatic ductal adenocarcinoma recruits regulatory T cells to avoid immune detection," *Journal of the American College of Surgeons*, vol. 206, no. 5, pp. 849–854, 2008.
- [76] A. Okamoto, T. Nikaido, K. Ochiai et al., "Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells," *Clinical Cancer Research*, vol. 11, no. 16, pp. 6030–6039, 2005.
- [77] G. Brandacher, A. Perathoner, R. Ladurner et al., "Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells," *Clinical Cancer Research*, vol. 12, no. 4, pp. 1144–1151, 2006.
- [78] A. Huang, D. Fuchs, B. Widner, C. Glover, D. C. Henderson, and T. G. Allen-Mersh, "Serum tryptophan decrease correlates with immune activation and impaired quality of life in colorectal cancer," *British Journal of Cancer*, vol. 86, no. 11, pp. 1691–1696, 2002.
- [79] S. Astigiano, B. Morandi, R. Costa et al., "Eosinophil granulocytes account for indoleamine 2,3-dioxygenase-mediated immune escape in human non-small cell lung cancer," *Neoplasia*, vol. 7, no. 4, pp. 390–396, 2005.
- [80] R. Riesenber, C. Weiler, O. Spring et al., "Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma," *Clinical Cancer Research*, vol. 13, no. 23, pp. 6993–7002, 2007.
- [81] X. Yao, C. N. Qian, Z. F. Zhang et al., "Two distinct types of blood vessels in clear cell renal cell carcinoma have contrasting prognostic implications," *Clinical Cancer Research*, vol. 13, no. 1, pp. 161–169, 2007.
- [82] J. R. Conejo-Garcia, F. Benencia, M. C. Courreges et al., "Tumor-infiltrating dendritic cell precursors recruited by a β -defensin contribute to vasculogenesis under the influence of Vegf-A," *Nature Medicine*, vol. 10, no. 9, pp. 950–958, 2004.
- [83] T. J. Curiel, P. Cheng, P. Mottram et al., "Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer," *Cancer Research*, vol. 64, no. 16, pp. 5535–5538, 2004.
- [84] A. P. Vicari, B. Vanbervliet, C. Massacrier et al., "In vivo manipulation of dendritic cell migration and activation to elicit antitumor immunity," *Novartis Foundation Symposium*, vol. 256, pp. 241–254, 2004.
- [85] S. Jung, D. Unutmaz, P. Wong et al., "In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens," *Immunity*, vol. 17, no. 2, pp. 211–220, 2002.
- [86] N. P. Restifo, Y. Kawakami, F. Marincola et al., "Molecular mechanisms used by tumors to escape immune recognition: immunogenotherapy and the cell biology of major histocompatibility complex class I," *Journal of Immunotherapy*, vol. 14, no. 3, pp. 182–190, 1993.
- [87] F. Cheng, H. W. Wang, A. Cuenca et al., "A critical role for Stat3 signaling in immune tolerance," *Immunity*, vol. 19, no. 3, pp. 425–436, 2003.
- [88] H. Ueno, E. Klechevsky, R. Morita et al., "Dendritic cell subsets in health and disease," *Immunological Reviews*, vol. 219, no. 1, pp. 118–142, 2007.
- [89] J. F. Bromberg, M. H. Wrzeszczynska, G. Devgan et al., "Stat3 as an oncogene," *Cell*, vol. 98, no. 3, pp. 295–303, 1999.
- [90] T. Wang, G. Niu, M. Kortylewski et al., "Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells," *Nature Medicine*, vol. 10, no. 1, pp. 48–54, 2004.
- [91] Y. Nefedova, M. Huang, S. Kusmartsev et al., "Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer," *Journal of Immunology*, vol. 172, no. 1, pp. 464–474, 2004.
- [92] S. J. Park, T. Nakagawa, H. Kitamura et al., "IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation," *Journal of Immunology*, vol. 173, no. 6, pp. 3844–3854, 2004.
- [93] Y. Nefedova, P. Cheng, M. Alsina, W. S. Dalton, and D. I. Gabrilovich, "Involvement of Notch-1 signaling in bone marrow stroma-mediated de novo drug resistance of myeloma and other malignant lymphoid cell lines," *Blood*, vol. 103, no. 9, pp. 3503–3510, 2004.
- [94] Y. Nefedova and D. I. Gabrilovich, "Targeting of Jak/STAT pathway in antigen presenting cells in cancer," *Current Cancer Drug Targets*, vol. 7, no. 1, pp. 71–77, 2007.
- [95] P. Cheng, J. Zhou, and D. Gabrilovich, "Regulation of dendritic cell differentiation and function by Notch and Wnt pathways," *Immunological Reviews*, vol. 234, no. 1, pp. 105–119, 2010.
- [96] H. Yu, M. Kortylewski, and D. Pardoll, "Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment," *Nature Reviews Immunology*, vol. 7, no. 1, pp. 41–51, 2007.
- [97] H. Yu, D. Pardoll, and R. Jove, "STATs in cancer inflammation and immunity: a leading role for STAT3," *Nature Reviews Cancer*, vol. 9, no. 11, pp. 798–809, 2009.
- [98] M. Kortylewski, M. Kujawski, T. Wang et al., "Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity," *Nature Medicine*, vol. 11, no. 12, pp. 1314–1321, 2005.
- [99] L. Burdelya, M. Kujawski, G. Niu et al., "Stat3 activity in melanoma cells affects migration of immune effector cells and nitric oxide-mediated antitumor effects," *Journal of Immunology*, vol. 174, no. 7, pp. 3925–3931, 2005.
- [100] K. Evel-Kabler, X. T. Song, M. Aldrich, X. F. Huang, and S. Y. Chen, "SOCS1 restricts dendritic cells' ability to break self tolerance and induce antitumor immunity by regulating IL-12 production and signaling," *Journal of Clinical Investigation*, vol. 116, no. 1, pp. 90–100, 2006.
- [101] E. M. Shevach, "CD4+CD25+ suppressor T cells: more questions than answers," *Nature Reviews Immunology*, vol. 2, no. 6, pp. 389–400, 2002.
- [102] S. Sakaguchi, "Regulatory T cells: key controllers of immunologic self-tolerance," *Cell*, vol. 101, no. 5, pp. 455–458, 2000.
- [103] S. Sakaguchi, "Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses," *Annual Review of Immunology*, vol. 22, pp. 531–562, 2004.
- [104] E. Suri-Payer, A. Z. Amar, A. M. Thornton, and E. M. Shevach, "CD4+CD25+ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells," *Journal of Immunology*, vol. 160, no. 3, pp. 1212–1218, 1998.
- [105] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells

- expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases,” *Journal of Immunology*, vol. 155, no. 3, pp. 1151–1164, 1995.
- [106] S. Sakaguchi, M. Miyara, C. M. Costantino, and D. A. Hafler, “FOXP3 + regulatory T cells in the human immune system,” *Nature Reviews Immunology*, vol. 10, no. 7, pp. 490–500, 2010.
- [107] H. Nishikawa and S. Sakaguchi, “Regulatory T cells in tumor immunity,” *International Journal of Cancer*, vol. 127, no. 4, pp. 759–767, 2010.
- [108] S. Sakaguchi, K. Wing, Y. Onishi, P. Prieto-Martin, and T. Yamaguchi, “Regulatory T cells: how do they suppress immune responses?” *International Immunology*, vol. 21, no. 10, pp. 1105–1111, 2009.
- [109] Y. Belkaid, C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks, “CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity,” *Nature*, vol. 420, no. 6915, pp. 502–507, 2002.
- [110] D. Q. Tran and E. M. Shevach, “Therapeutic potential of FOXP3+ regulatory T cells and their interactions with dendritic cells,” *Human Immunology*, vol. 70, no. 5, pp. 294–299, 2009.
- [111] S. Sakaguchi, “Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self,” *Nature Immunology*, vol. 6, no. 4, pp. 345–352, 2005.
- [112] J. D. Fontenot and A. Y. Rudensky, “A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3,” *Nature Immunology*, vol. 6, no. 4, pp. 331–337, 2005.
- [113] W. Liu, A. L. Putnam, Z. Xu-Yu et al., “CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells,” *Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1701–1711, 2006.
- [114] N. Seddiki, B. Santner-Nanan, J. Martinson et al., “Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells,” *Journal of Experimental Medicine*, vol. 203, no. 7, pp. 1693–1700, 2006.
- [115] F. Simonetta, A. Chiali, C. Cordier et al., “Increased CD127 expression on activated FOXP3+CD4+ regulatory T cells,” *European Journal of Immunology*, vol. 40, no. 9, pp. 2528–2538, 2010.
- [116] G. Borsellino, M. Kleinewietfeld, D. di Mitri et al., “Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression,” *Blood*, vol. 110, no. 4, pp. 1225–1232, 2007.
- [117] S. Deaglio, K. M. Dwyer, W. Gao et al., “Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression,” *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1257–1265, 2007.
- [118] J. D. Fontenot, M. A. Gavin, and A. Y. Rudensky, “Foxp3 programs the development and function of CD4+CD25+ regulatory T cells,” *Nature Immunology*, vol. 4, no. 4, pp. 330–336, 2003.
- [119] J. D. Fontenot, J. P. Rasmussen, L. M. Williams, J. L. Dooley, A. G. Farr, and A. Y. Rudensky, “Regulatory T cell lineage specification by the forkhead transcription factor Foxp3,” *Immunity*, vol. 22, no. 3, pp. 329–341, 2005.
- [120] M. Kronenberg and A. Rudensky, “Regulation of immunity by self-reactive T cells,” *Nature*, vol. 435, no. 7042, pp. 598–604, 2005.
- [121] S. Hori, T. Nomura, and S. Sakaguchi, “Control of regulatory T cell development by the transcription factor Foxp3,” *Science*, vol. 299, no. 5609, pp. 1057–1061, 2003.
- [122] M. Asano, M. Toda, N. Sakaguchi, and S. Sakaguchi, “Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation,” *Journal of Experimental Medicine*, vol. 184, no. 2, pp. 387–396, 1996.
- [123] C. L. Bennett, J. Christie, F. Ramsdell et al., “The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3,” *Nature Genetics*, vol. 27, no. 1, pp. 20–21, 2001.
- [124] M. E. Brunkow, E. W. Jeffery, K. A. Hjerrild et al., “Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse,” *Nature Genetics*, vol. 27, no. 1, pp. 68–73, 2001.
- [125] S. Sakaguchi, K. Wing, and M. Miyara, “Regulatory T cells—a brief history and perspective,” *European Journal of Immunology*, vol. 37, supplement 1, pp. S116–S123, 2007.
- [126] B. Valzasina, S. Piconese, C. Guiducci, and M. P. Colombo, “Tumor-induced expansion of regulatory T cells by conversion of CD4+CD25- lymphocytes is thymus and proliferation independent,” *Cancer Research*, vol. 66, no. 8, pp. 4488–4495, 2006.
- [127] T. J. Curiel, G. Coukos, L. Zou et al., “Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival,” *Nature Medicine*, vol. 10, no. 9, pp. 942–949, 2004.
- [128] N. Janikashvili, C. J. LaCasse, C. Larmonier et al., “Allogeneic effector/memory Th-1 cells impair FoxP3+ regulatory T lymphocytes and synergize with chaperone-rich cell lysate vaccine to treat leukemia,” *Blood*, vol. 117, no. 5, pp. 1555–1564, 2011.
- [129] C. V. Nicchitta, “Biochemical, cell biological and immunological issues surrounding the endoplasmic reticulum chaperone GRP94/gp96,” *Current Opinion in Immunology*, vol. 10, no. 1, pp. 103–109, 1998.
- [130] E. M. Shevach, “From vanilla to 28 flavors: multiple varieties of T regulatory cells,” *Immunity*, vol. 25, no. 2, pp. 195–201, 2006.
- [131] E. M. Shevach, R. A. DiPaolo, J. Andersson, D. M. Zhao, G. L. Stephens, and A. M. Thornton, “The lifestyle of naturally occurring CD4+CD25+Foxp3+ regulatory T cells,” *Immunological Reviews*, vol. 212, pp. 60–73, 2006.
- [132] E. M. Shevach and G. L. Stephens, “The GITR-GITRL interaction: co-stimulation or contrasuppression of regulatory activity?” *Nature Reviews Immunology*, vol. 6, no. 8, pp. 613–618, 2006.
- [133] S. Geng, Y. Yu, Y. Kang et al., “Efficient induction of CD25-iTreg by co-immunization requires strongly antigenic epitopes for T cells,” *BMC Immunology*, vol. 12, p. 27, 2011.
- [134] O. Annacker, R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano, and A. Bandeira, “CD25+ CD4+ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10,” *Journal of Immunology*, vol. 166, no. 5, pp. 3008–3018, 2001.
- [135] J. Lehmann, J. Huehn, M. de la Rosa et al., “Expression of the integrin $\alpha E\beta 7$ identifies unique subsets of CD25+ as well as CD25- regulatory T cells,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 20, pp. 13031–13036, 2002.
- [136] S. Zelenay, T. Lopes-Carvalho, I. Caramalho, M. F. Moraes-Fontes, M. Rebelo, and J. Demengeot, “Foxp3+ CD25- CD4

- T cells constitute a reservoir of committed regulatory cells that regain CD25 expression upon homeostatic expansion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 11, pp. 4091–4096, 2005.
- [137] K. Nakamura, A. Kitani, I. Fuss et al., "TGF- β 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice," *Journal of Immunology*, vol. 172, no. 2, pp. 834–842, 2004.
- [138] K. Nakamura, A. Kitani, and W. Strober, "Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor β ," *Journal of Experimental Medicine*, vol. 194, no. 5, pp. 629–644, 2001.
- [139] C. A. Piccirillo, J. J. Letterio, A. M. Thornton et al., "CD4+CD25+ regulatory T cells can mediate suppressor function in the absence of transforming growth factor β 1 production and responsiveness," *Journal of Experimental Medicine*, vol. 196, no. 2, pp. 237–245, 2002.
- [140] A. M. Thornton and E. M. Shevach, "CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production," *Journal of Experimental Medicine*, vol. 188, no. 2, pp. 287–296, 1998.
- [141] C. Asseman, S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie, "An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation," *Journal of Experimental Medicine*, vol. 190, no. 7, pp. 995–1004, 1999.
- [142] L. W. Collison, C. J. Workman, T. T. Kuo et al., "The inhibitory cytokine IL-35 contributes to regulatory T-cell function," *Nature*, vol. 450, no. 7169, pp. 566–569, 2007.
- [143] S. Read, V. Malmström, and F. Powrie, "Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25+CD4+ regulatory cells that control intestinal inflammation," *Journal of Experimental Medicine*, vol. 192, no. 2, pp. 295–302, 2000.
- [144] L. Cederbom, H. Hall, and F. Ivars, "CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells," *European Journal of Immunology*, vol. 30, no. 6, pp. 1538–1543, 2000.
- [145] T. Bopp, C. Becker, M. Klein et al., "Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression," *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1303–1310, 2007.
- [146] D. C. Gondek, L. F. Lu, S. A. Quezada, S. Sakaguchi, and R. J. Noelle, "Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism," *Journal of Immunology*, vol. 174, no. 4, pp. 1783–1786, 2005.
- [147] W. J. Grossman, J. W. Verbsky, W. Barchet, M. Colonna, J. P. Atkinson, and T. J. Ley, "Human T regulatory cells can use the perforin pathway to cause autologous target cell death," *Immunity*, vol. 21, no. 4, pp. 589–601, 2004.
- [148] D. M. Zhao, A. M. Thornton, R. J. DiPaolo, and E. M. Shevach, "Activated CD4+CD25+ T cells selectively kill B lymphocytes," *Blood*, vol. 107, no. 10, pp. 3925–3932, 2006.
- [149] S. F. Hussain and Y. Paterson, "CD4+CD25+ regulatory T cells that secrete TGF β and IL-10 are preferentially induced by a vaccine vector," *Journal of Immunotherapy*, vol. 27, no. 5, pp. 339–346, 2004.
- [150] F. Ghiringhelli, N. Larmonier, E. Schmitt et al., "CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative," *European Journal of Immunology*, vol. 34, no. 2, pp. 336–344, 2004.
- [151] U. K. Liyanage, T. T. Moore, H. G. Joo et al., "Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma," *Journal of Immunology*, vol. 169, no. 5, pp. 2756–2761, 2002.
- [152] M. Viguier, F. Lemaître, O. Verola et al., "Foxp3 expressing CD4+CD25high regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells," *Journal of Immunology*, vol. 173, no. 2, pp. 1444–1453, 2004.
- [153] R. W. Griffiths, E. Elkord, D. E. Gilham et al., "Frequency of regulatory T cells in renal cell carcinoma patients and investigation of correlation with survival," *Cancer Immunology, Immunotherapy*, vol. 56, no. 11, pp. 1743–1753, 2007.
- [154] M. P. Colombo and S. Piconese, "Regulatory T-cell inhibition versus depletion: the right choice in cancer immunotherapy," *Nature Reviews Cancer*, vol. 7, no. 11, pp. 880–887, 2007.
- [155] A. Iellem, M. Mariani, R. Lang et al., "Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4+CD25+ regulatory T cells," *Journal of Experimental Medicine*, vol. 194, no. 6, pp. 847–853, 2001.
- [156] D. A. A. Vignali, L. W. Collison, and C. J. Workman, "How regulatory T cells work," *Nature Reviews Immunology*, vol. 8, no. 7, pp. 523–532, 2008.
- [157] L. Zou, B. Barnett, H. Safah et al., "Bone marrow is a reservoir for CD4+CD25+ regulatory T cells that traffic through CXCL12/CXCR4 signals," *Cancer Research*, vol. 64, no. 22, pp. 8451–8455, 2004.
- [158] E. Y. Woo, C. S. Chu, T. J. Goletz et al., "Regulatory CD4+CD25+ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer," *Cancer Research*, vol. 61, no. 12, pp. 4766–4772, 2001.
- [159] E. Y. Woo, H. Yeh, C. S. Chu et al., "Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation," *Journal of Immunology*, vol. 168, no. 9, pp. 4272–4276, 2002.
- [160] H. Nishikawa, E. Jäger, G. Ritter, L. J. Old, and S. Gnjatich, "CD4+ CD25+ regulatory T cells control the induction of antigen-specific CD4+ helper T cell responses in cancer patients," *Blood*, vol. 106, no. 3, pp. 1008–1011, 2005.
- [161] J. Shimizu, S. Yamazaki, and S. Sakaguchi, "Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity," *Journal of Immunology*, vol. 163, no. 10, pp. 5211–5218, 1999.
- [162] R. Somasundaram, L. Jacob, R. Swoboda et al., "Inhibition of cytolytic T lymphocyte proliferation by autologous CD4+/CD25+ regulatory T cells in a colorectal carcinoma patient is mediated by transforming growth factor- β ," *Cancer Research*, vol. 62, no. 18, pp. 5267–5272, 2002.
- [163] R. P. M. Suttmuller, L. M. van Duivenvoorde, A. van Elsas et al., "Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25+ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses," *Journal of Experimental Medicine*, vol. 194, no. 6, pp. 823–832, 2001.
- [164] F. Ghiringhelli, C. Ménard, M. Terme et al., "CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor- β -dependent manner," *Journal of Experimental Medicine*, vol. 202, no. 8, pp. 1075–1085, 2005.

- [165] N. Larmonier, J. Cantrell, C. LaCasse et al., "Chaperone-rich tumor cell lysate-mediated activation of antigen-presenting cells resists regulatory T cell suppression," *Journal of Leukocyte Biology*, vol. 83, no. 4, pp. 1049–1059, 2008.
- [166] N. Misra, J. Bayry, S. Lacroix-Desmazes, M. D. Kazatchkine, and S. V. Kaveri, "Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells," *Journal of Immunology*, vol. 172, no. 8, pp. 4676–4680, 2004.
- [167] K. Sato, S. Tateishi, K. Kubo, T. Mimura, K. Yamamoto, and H. Kanda, "Downregulation of IL-12 and a novel negative feedback system mediated by CD25+CD4+ T cells," *Biochemical and Biophysical Research Communications*, vol. 330, no. 1, pp. 226–232, 2005.
- [168] L. S. Taams, J. M. R. van Amelsfort, M. M. Tiemessen et al., "Modulation of monocyte/macrophage function by human CD4+CD25+ regulatory T cells," *Human Immunology*, vol. 66, no. 3, pp. 222–230, 2005.
- [169] S. J. Prasad, K. J. Farrand, S. A. Matthews, J. H. Chang, R. S. McHugh, and F. Ronchese, "Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4+CD25+ regulatory T cells," *Journal of Immunology*, vol. 174, no. 1, pp. 90–98, 2005.
- [170] P. Yu, Y. Lee, W. Liu et al., "Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors," *Journal of Experimental Medicine*, vol. 201, no. 5, pp. 779–791, 2005.
- [171] N. Larmonier, N. Janikashvili, C. J. Lacasse et al., "Imatinib mesylate inhibits CD4+CD25+ regulatory T cell activity and enhances active immunotherapy against BCR-ABL- tumors," *Journal of Immunology*, vol. 181, no. 10, pp. 6955–6963, 2008.
- [172] A. El Andaloussi, Y. U. Han, and M. S. Lesniak, "Prolongation of survival following depletion of CD4+CD25+ regulatory T cells in mice with experimental brain tumors," *Journal of Neurosurgery*, vol. 105, no. 3, pp. 430–437, 2006.
- [173] A. Comes, O. Rosso, A. M. Orengo et al., "CD25+ regulatory T cell depletion augments immunotherapy of micrometastases by an IL-21-secreting cellular vaccine," *Journal of Immunology*, vol. 176, no. 3, pp. 1750–1758, 2006.
- [174] P. Attia, D. J. Powell Jr., A. V. Maker, R. J. Kreitman, I. Pastan, and S. A. Rosenberg, "Selective elimination of human regulatory T lymphocytes in vitro with the recombinant immunotoxin LMB-2," *Journal of Immunotherapy*, vol. 29, no. 2, pp. 208–214, 2006.
- [175] J. Dannull, Z. Su, D. Rizzieri et al., "Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells," *Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3623–3633, 2005.
- [176] K. Ko, S. Yamazaki, K. Nakamura et al., "Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells," *Journal of Experimental Medicine*, vol. 202, no. 7, pp. 885–891, 2005.
- [177] T. Ramirez-Montagut, A. Chow, D. Hirschhorn-Cymerman et al., "Glucocorticoid-induced TNF receptor family related gene activation overcomes tolerance/ignorance to melanoma differentiation antigens and enhances antitumor immunity," *Journal of Immunology*, vol. 176, no. 11, pp. 6434–6442, 2006.
- [178] S. Read, R. Greenwald, A. Izcue et al., "Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo," *Journal of Immunology*, vol. 177, no. 7, pp. 4376–4383, 2006.
- [179] S. A. Quezada, K. S. Peggs, M. A. Curran, and J. P. Allison, "CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 1935–1945, 2006.
- [180] A. J. Muller, J. B. DuHadaway, P. S. Donover, E. Sutanto-Ward, and G. C. Prendergast, "Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy," *Nature Medicine*, vol. 11, no. 3, pp. 312–319, 2005.
- [181] L. Gorelink and R. A. Flavell, "Immune-mediated eradication of tumors through the blockade of transforming growth factor- β signaling in T cells," *Nature Medicine*, vol. 7, no. 10, pp. 1118–1122, 2001.
- [182] D. A. Thomas and J. Massagué, "TGF- β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance," *Cancer Cell*, vol. 8, no. 5, pp. 369–380, 2005.
- [183] M. E. C. Lutsiak, R. T. Semnani, R. de Pascalis, S. V. S. Kashmiri, J. Schlom, and H. Sabzevari, "Inhibition of CD4+25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide," *Blood*, vol. 105, no. 7, pp. 2862–2868, 2005.
- [184] J. Taieb, N. Chaput, N. Scharzt et al., "Chemoimmunotherapy of tumors: cyclophosphamide synergizes with exosome based vaccines," *Journal of Immunology*, vol. 176, no. 5, pp. 2722–2729, 2006.
- [185] B. Valzasina, C. Guiducci, H. Dislich, N. Killeen, A. D. Weinberg, and M. P. Colombo, "Triggering of OX40 (CD134) on CD4+CD25+ T cells blocks their inhibitory activity: a novel regulatory role for OX40 and its comparison with GITR," *Blood*, vol. 105, no. 7, pp. 2845–2851, 2005.
- [186] G. Peng, Z. Guo, Y. Kiniwa et al., "Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function," *Science*, vol. 309, no. 5739, pp. 1380–1384, 2005.
- [187] F. Ghiringhelli, C. Menard, P. E. Puig et al., "Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients," *Cancer Immunology, Immunotherapy*, vol. 56, no. 5, pp. 641–648, 2007.
- [188] Y. Motoyoshi, K. Kaminoda, O. Saitoh et al., "Different mechanisms for anti-tumor effects of low- and high-dose cyclophosphamide," *Oncology Reports*, vol. 16, no. 1, pp. 141–146, 2006.
- [189] S. Audia, A. Nicolas, D. Cathelin et al., "Increase of CD4+CD25+ regulatory T cells in the peripheral blood of patients with metastatic carcinoma: a phase I clinical trial using cyclophosphamide and immunotherapy to eliminate CD4+CD25+ T lymphocytes," *Clinical and Experimental Immunology*, vol. 150, no. 3, pp. 523–530, 2007.
- [190] K. Mahnke, T. S. Johnson, S. Ring, and A. H. Enk, "Tolerogenic dendritic cells and regulatory T cells: a two-way relationship," *Journal of Dermatological Science*, vol. 46, no. 3, pp. 159–167, 2007.
- [191] S. Yamazaki, M. Patel, A. Harper et al., "Effective expansion of alloantigen-specific Foxp3+ CD25+ CD4+ regulatory T cells by dendritic cells during the mixed leukocyte reaction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 8, pp. 2758–2763, 2006.
- [192] M. V. Dhodapkar and R. M. Steinman, "Antigen-bearing immature dendritic cells induce peptide-specific CD8+ regulatory T cells in vivo in humans," *Blood*, vol. 100, no. 1, pp. 174–177, 2002.

- [193] K. Sato, N. Yamashita, M. Baba, and T. Matsuyama, "Modified myeloid dendritic cells act as regulatory dendritic cells to induce anergic and regulatory T cells," *Blood*, vol. 101, no. 9, pp. 3581–3589, 2003.
- [194] M. Gilliet and Y. J. Liu, "Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells," *Journal of Experimental Medicine*, vol. 195, no. 6, pp. 695–704, 2002.
- [195] S. Wei, I. Kryczek, L. Zou et al., "Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma," *Cancer Research*, vol. 65, no. 12, pp. 5020–5026, 2005.
- [196] S. Yamazaki, T. Iyoda, K. Tarbell et al., "Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells," *Journal of Experimental Medicine*, vol. 198, no. 2, pp. 235–247, 2003.
- [197] A. Wakkach, N. Fournier, V. Brun, J. P. Breittmayer, F. Cottrez, and H. Groux, "Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo," *Immunity*, vol. 18, no. 5, pp. 605–617, 2003.
- [198] P. Serra, A. Amrani, J. Yamanouchi et al., "CD40 ligation releases immature dendritic cells from the control of regulatory CD4+CD25+ T cells," *Immunity*, vol. 19, no. 6, pp. 877–889, 2003.
- [199] A. Ouabed, F. X. Hubert, D. Chabannes, L. Gautreau, M. Heslan, and R. Josien, "Differential control of T regulatory cell proliferation and suppressive activity by mature plasmacytoid versus conventional spleen dendritic cells," *Journal of Immunology*, vol. 180, no. 9, pp. 5862–5870, 2008.
- [200] M. B. Lutz and G. Schuler, "Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity?" *Trends in Immunology*, vol. 23, no. 9, pp. 445–449, 2002.
- [201] K. Mahnke and A. H. Enk, "Dendritic cells: key cells for the induction of regulatory T cells?" *Current Topics in Microbiology and Immunology*, vol. 293, pp. 133–150, 2005.
- [202] V. Verhasselt, O. Vosters, C. Beuneu, C. Nicaise, P. Stordeur, and M. Goldman, "Induction of FOXP3-expressing regulatory CD4pos T cells by human mature autologous dendritic cells," *European Journal of Immunology*, vol. 34, no. 3, pp. 762–772, 2004.
- [203] Y. Belkaid and G. Oldenhove, "Tuning microenvironments: induction of regulatory T cells by dendritic cells," *Immunity*, vol. 29, no. 3, pp. 362–371, 2008.
- [204] A. Langenkamp, M. Messi, A. Lanzavecchia, and F. Sallusto, "Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells," *Nature Immunology*, vol. 1, no. 4, pp. 311–316, 2000.
- [205] D. H. Munn, M. D. Sharma, J. R. Lee et al., "Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase," *Science*, vol. 297, no. 5588, pp. 1867–1870, 2002.
- [206] F. Fallarino, U. Grohmann, K. W. Hwang et al., "Modulation of tryptophan catabolism by regulatory T cells," *Nature Immunology*, vol. 4, no. 12, pp. 1206–1212, 2003.
- [207] M. D. Sharma, D. Y. Hou, Y. Liu et al., "Indoleamine 2,3-dioxygenase controls conversion of Foxp3+ Tregs to TH17-like cells in tumor-draining lymph nodes," *Blood*, vol. 113, no. 24, pp. 6102–6111, 2009.
- [208] B. M. Matta, A. Castellaneta, and A. W. Thomson, "Tolerogenic plasmacytoid DC," *European Journal of Immunology*, vol. 40, no. 10, pp. 2667–2676, 2010.
- [209] M. L. Belladonna, U. Grohmann, P. Guidetti et al., "Kynurenine pathway enzymes in dendritic cells initiate tolerogenesis in the absence of functional IDO," *Journal of Immunology*, vol. 177, no. 1, pp. 130–137, 2006.
- [210] F. Fallarino, U. Grohmann, S. You et al., "The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor ζ -chain and induce a regulatory phenotype in naive T cells," *Journal of Immunology*, vol. 176, no. 11, pp. 6752–6761, 2006.
- [211] W. J. Chen and S. M. Wahl, "TGF- β : the missing link in CD4+CD25+ regulatory T cell-mediated immunosuppression," *Cytokine and Growth Factor Reviews*, vol. 14, no. 2, pp. 85–89, 2003.
- [212] W. Ge, X. Ma, X. Li et al., "B7-H1 up-regulation on dendritic-like leukemia cells suppresses T cell immune function through modulation of IL-10/IL-12 production and generation of Treg cells," *Leukemia Research*, vol. 33, no. 7, pp. 948–957, 2009.
- [213] A. J. Coyle and J. C. Gutierrez-Ramos, "The expanding B7 superfamily: increasing complexity in costimulatory signals regulating T cell function," *Nature Immunology*, vol. 2, no. 3, pp. 203–209, 2001.
- [214] T. J. Curiel, S. Wei, H. Dong et al., "Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity," *Nature Medicine*, vol. 9, no. 5, pp. 562–567, 2003.
- [215] N. Selenko-Gebauer, O. Majdic, A. Szekeres et al., "B7-H1 (programmed death-1 ligand) on dendritic cells is involved in the induction and maintenance of T cell anergy," *Journal of Immunology*, vol. 170, no. 7, pp. 3637–3644, 2003.
- [216] S. Wei, I. Kryczek, and W. Zou, "Regulatory T-cell compartmentalization and trafficking," *Blood*, vol. 108, no. 2, pp. 426–431, 2006.
- [217] T. Ishida, T. Ishii, A. Inagaki et al., "Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege," *Cancer Research*, vol. 66, no. 11, pp. 5716–5722, 2006.
- [218] M. Gobert, I. Treilleux, N. Bendriss-Vermare et al., "Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome," *Cancer Research*, vol. 69, no. 5, pp. 2000–2009, 2009.
- [219] R. Godiska, D. Chantry, C. J. Raport et al., "Human macrophage-derived chemokine (MDC), a novel chemoattractant for monocytes, monocyte-derived dendritic cells, and natural killer cells," *Journal of Experimental Medicine*, vol. 185, no. 9, pp. 1595–1604, 1997.
- [220] M. Vulcano, C. Albanesi, A. Stoppacciaro et al., "Dendritic cells as a major source of macrophage-derived chemokine/CCL22 in vitro and in vivo," *European Journal of Immunology*, vol. 31, no. 3, pp. 812–822, 2001.
- [221] K. Enarsson, A. Lundgren, B. Kindlund et al., "Function and recruitment of mucosal regulatory T cells in human chronic *Helicobacter pylori* infection and gastric adenocarcinoma," *Clinical Immunology*, vol. 121, no. 3, pp. 358–368, 2006.
- [222] R. Houot, I. Perrot, E. Garcia, I. Durand, and S. Lebecque, "Human CD4+CD25high regulatory T cells modulate myeloid but not plasmacytoid dendritic cells activation," *Journal of Immunology*, vol. 176, no. 9, pp. 5293–5298, 2006.
- [223] S. Sakaguchi, M. Ono, R. Setoguchi et al., "Foxp3+CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease," *Immunological Reviews*, vol. 212, pp. 8–27, 2006.
- [224] I. Kryczek, L. Zou, P. Rodriguez et al., "B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma," *Journal of Experimental Medicine*, vol. 203, no. 4, pp. 871–881, 2006.

- [225] W. K. Suh, B. U. Gajewska, H. Okada et al., "The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses," *Nature Immunology*, vol. 4, no. 9, pp. 899–906, 2003.
- [226] I. Kryczek, S. Wei, L. Zou et al., "Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells," *Journal of Immunology*, vol. 177, no. 1, pp. 40–44, 2006.
- [227] M. Sarris, K. G. Andersen, F. Randow, L. Mayr, and A. G. Betz, "Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition," *Immunity*, vol. 28, no. 3, pp. 402–413, 2008.



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