

Infiltrating Treg Cells Suppress Anti-Tumor Immunity in Tumor Microenvironment

Tania Sarkar, Gaurisankar Sa*

Division of Molecular Medicine, Bose Institute, P-1/12, CIT Road, Scheme VIII, Kolkata, WB, 700054, India

*Correspondence: Gaurisankar Sa, Division of Molecular Medicine, Bose Institute, P-1/12, CIT Scheme VII M, Kolkata-700054, India. Tel: +91-33-2569-3258; Fax: +91-33-2355-3886; E-mail: gauri@jcbose.ac.in

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Abstract

Avoidance of immune-surveillance by tumor is one of the important hallmarks of cancer and tumor microenvironment adapts host immune system in favor of this immune-escape mechanism. One of the major immune components that is responsible for immune-escape of tumor is T-regulatory cell. Tregs are found to be elevated in the peripheral circulation of tumor patient and from the circulation they infiltrate to the tumor-site which depends on the interaction between chemokines and chemokine receptors. Infiltrated Tregs are potent immunosuppressors and foster tumor growth by suppressing the effectiveness of helper T cells, NK cells, dendritic cells and macrophages making a tolerogenic microenvironment in the tumor-site. Rapid regeneration of infiltrating Tregs in the tumor milieu aids to this process. Combined action of suppression and survival of tumor infiltrating Tregs in tumor microenvironment makes them a major stumbling block in the battle against cancer.

Keywords: Treg cells; Tumor microenvironment; Inhibit antitumor immune; Anti-tumor

ABBREVIATIONS

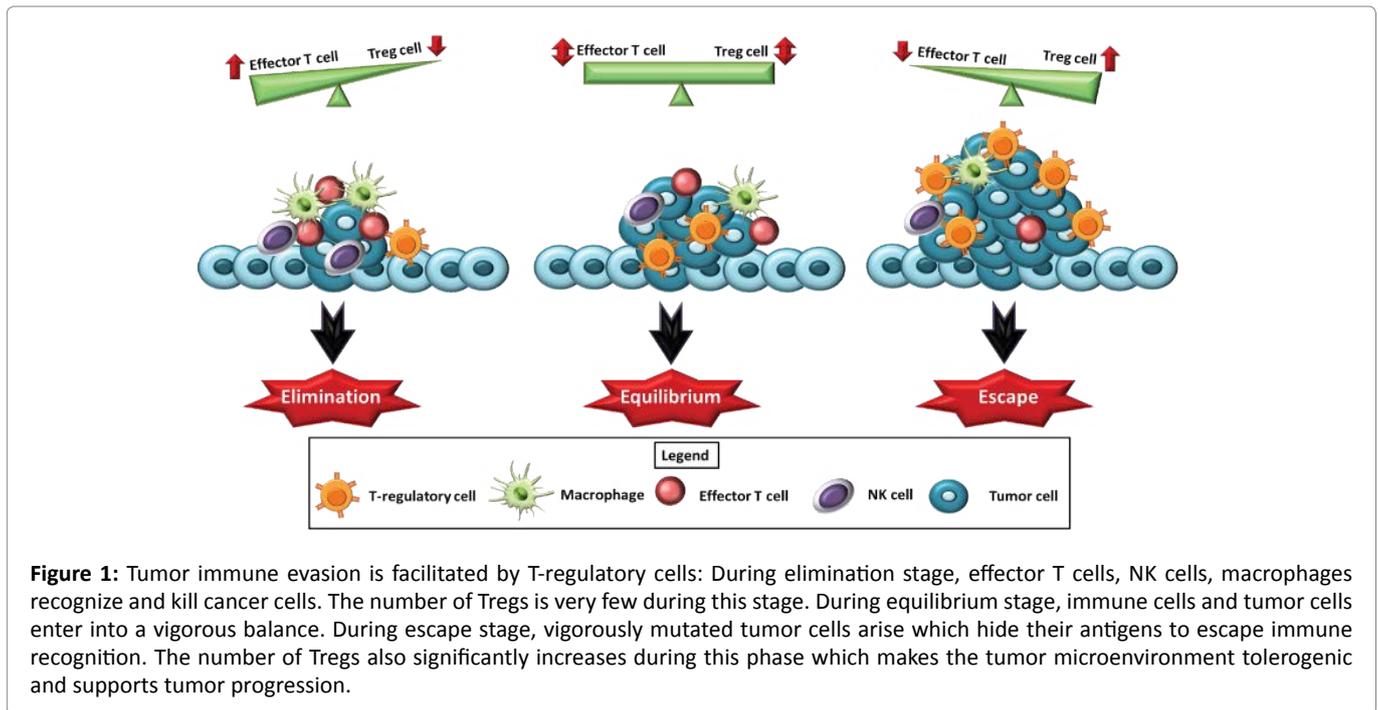
Treg: Tregulatory cell; Teff cell: T effector cell; TME: Tumor microenvironment; CCR/ CXCR: CC motif chemokine receptor/ CXC motif chemokine receptor; CCL/ CXCL: CC motif chemokine ligand/ CXC motif chemokine ligand; NK cell: Natural killer cell; DC: Dendritic cell; APC: Antigen-presenting cell; FOXP3: Fork-head box P3; TGFβ: Transforming growth factor-β; IL: Interleukin

INTRODUCTION

The interaction of innate and adaptive immune system is a decisive factor for identification and effective elimination of foreign microorganisms as well as tumor development in the host body [1]. But till date it has been an enigmatic issue, how tumor develops regardless of this immune-surveillance mechanism in our body. The correlation between immune system and tumorigenesis has long been established by Rudolf Virchow in 1863. He first anticipated the relationship between immune cell infiltration and cancer development. Later the theory was confirmed by several scientific discoveries and it has been confirmed that tumorigenesis is a complex and dynamic process and tumor microenvironment (TME) works hand-in-hand with growing tumor cells to favor tumor development. They guide body's immune

system to behave in a certain way, i.e., secretion of immunosuppressive cytokines, inactivation of dendritic cells and macrophages, generation of tumor-associated macrophages, building Th2 cytokine biasness. All these actions can be summed up under the name of tumor-immunoediting; a well-accepted theory by Robert Schreiber (2003) which consists of three stages: (i) Elimination, (ii) Equilibrium and (iii) Escape; each step involves adaptation of immune cells in favor of tumor progression (Figure 1) [2].

During elimination phase antigen processing cells (dendritic cells, macrophages etc.) secrete inflammatory cytokines, recognize tumor-associated antigens and eradicate emerging tumor thus defending host against cancer. During this stage CD4⁺ helper T cell, CD8⁺ T cytotoxic T cells, NK cells and macrophages eliminate tumor cells. During equilibrium the number of tumor cells and immune cells enter into a phase of vigorous poise. At this stage T-cell mediated immune response governs the tumor prognosis. In escape phase, tumor cells outnumber immune cells and ever more mutations accumulate [3,4]. Tumor cells hide their antigens which causes the failure of recognition and killing of tumor cells by immune system. Consistently mutated tumor variants that escape from immune selection pressure develop into extremely drug-



resistant, metastatic and aggressive tumors by dodging the immune-surveillance [5,6]. Avoidance of immune-surveillance is an important feature of tumor growth and it has been added as one of the significant hallmarks of cancer by Douglas Hanahan and Robert A. Weinberg (2011). Out of the various subsets of T cells, T-regulatory cells (Treg) are the key players that play pivotal role in this immune escape of tumor. CD4⁺CD25⁺FOXP3⁺ Treg cell lineage is requisite for induction of T cell tolerance and maintains autoimmunity. This feature of Tregs is one of the mechanisms of tumor immune evasion [7] (Figure 1).

By secretion of immunosuppressive cytokines Tregs create Th2-biased milieu which is essential for successful tumor promotion. Increased levels of Treg cells are found in peripheral blood and in tumor tissue of cancer patients and a high density of Tregs are correlated with poor outcome. Tregs found in the blood and tumor tissue of cancer patients suppress T-effector (Teff) cell and NK cell responses, interfering with both acquired and innate immunity [8]. Studies suggest that Tregs infiltrate from peripheral circulation and immune compartments to the tumor site and creates an environment suitable for tumor progression; hence they are major hurdle in cancer treatment [9]. Infiltration of lymphocytes depends on the interaction of chemokines and chemokine receptors on lymphocyte surface. Tregs express a plethora of chemokine receptor that responds to the chemokines secreted from the growing tumor mass. The signaling cascade involved with chemokines play essential roles in migration of Tregs in TME. The cumulative effect of

infiltration and rapid regeneration allows accumulation and sustenance of Tregs and makes the TME pro-tumorigenic in nature. Reports suggest that high Treg infiltrated breast tumors are extremely metastatic, have decreased survival rate and poor patient prognosis.

Recruitment and proliferation of Tregs in TME has gained clinical significance over the years and is a growing field of research. Monoclonal antibodies and drugs have been designed these days to selectively prevent the infiltration of Tregs in TME and some of these are in preclinical and clinical studies. This manuscript offers a comprehensive overview of the recent scientific studies in the areas of Treg infiltration, molecules involved behind this and its suppressive nature in favor of tumorigenesis.

TUMOR MICROENVIRONMENT AND TREG CELLS

Tumor microenvironment (TME) is the niche that surrounds the tumor mass and plays the central role in tumorigenesis. It consists of heterogeneous cell populations like tumor cells, stromal cells, fibroblasts, adipose cells, blood vessels, lymphatic cells, immune cells and inflammatory cells [10]. Other than that, it also contains a variety of non-cellular components like signaling molecules, growth factors, cytokines and cell-secreted factors. The combined interplay of these cells and molecules eventually determines the outcome of the tumor. TME commands the aberrant tissue functions and sends either growth-promoting or growth-inhibitory signals to the tumor cells depending upon the physiological state of an individual [11]. Growing tumor mass constantly

interact with the TME for accessibility of nutrients and growth signals. This interaction between tumor cells and the TME results in the evolution of more persistently mutated and therapy-resistant tumor cells. Immune cells contribute a paramount section of TME. CD4⁺ T cells, CD8⁺ T cells, B cells and NK cells constitute the bulk of anti-tumorigenic inflammatory cells and their relentless combat helps in the eradication of tumor cells [12]. On the other hand, existence of certain anti-inflammatory cells like Treg cells, tumor-associated macrophages and myeloid derived suppressor cells, T-regulatory cells in the TME makes this battle a challenging one [13,14]. Among them T-regulatory cells is one of the fundamental determinants of tumor growth [15]. During the equilibrium stage the critical balance between Treg cells versus Teff cells is the most compelling factor that ultimately determines the progression of cancer.

Tregs are a subclass of T cells that mediate peripheral tolerance. Tregs robustly suppress the activity of immune cells after an immune reaction in our body. Tregs have manifold roles in autoimmunity, fetal-maternal tolerance and transplantation tolerance. There are two types of Tregs that can be found in our body [16]. Natural Tregs (nTregs), develop in the thymus by stimulation of self-antigens. On the other hand, Tregs can also develop by specific stimulation in peripheral circulation. These Tregs are known as peripheral Treg cells (pTregs) or induced Treg cells (iTregs). Likewise, developing tumor cells modify our immune system to generate an enormous number of Tregs. These Tregs originate from the existing T-cells under specific cytokine stimulations [17]. Tregs generated during tumor condition are known as tumor Tregs (tTregs). iTreg cells/ tTregs phenotypically resemble iTregs, and both of them have similar phenotypic characteristics [18]. Tregs suppress the immune response through different mechanisms. Treg suppressive function is entirely dependent upon X-chromosome encoded transcription factor FOXP3 (Fork-head F-box protein) [19]. Treg cell functioning requires the presence of lineage-specification factor FOXP3. FOXP3 undertakes its manifold suppressive activities through transcriptional regulation of its target genes, acting either as transcriptional activator or repressor [20]. TGFβ is a strategic controller of the signaling pathways that regulates FOXP3 expression in CD4⁺CD25⁻ precursors which ultimately generates Treg cells [21,22]. Uninterrupted FOXP3 expression is compulsory for exploitive action of Treg cells. Tregs require consumption of IL2 from the surrounding environment for their vitality and uptake IL2 through IL2R receptor present of the cell surface. Hence, IL2R (CD25) also plays an imperative role in the generation of Tregs [23]. Tregs are typically represented by CD4⁺CD25⁺FOXP3⁺ cells. Tregs

are dual-faced in nature; on one hand they are beneficial by suppressing autoimmunity. On the other hand this immunosuppressive action can be detrimental in case of tumor condition. As T-regulatory cells are repressive by nature they silence the necessary inflammatory immune reactions against tumor. Subsequent deficiency of anti-tumorigenic cells in the TME in-turn favors the tumor growth. Several studies suggest that Tregs are found in higher amount in different stages of tumor patients. Tregs are found to be elevated not only in the peripheral circulation of tumor patient but also has been seen to be increased in tumor tissue in substantial amount [24]. It has been assumed that these Tregs infiltrate from circulation to tumor site where they create tolerogenic environment which is advantageous for tumor progress. Immune evasion of tumor cells is facilitated by high infiltration of Treg cells in the TME. Accumulation of Tregs in TME can occur by different mechanisms; firstly, by infiltration from peripheral circulation and secondly by proliferation of these infiltrated Tregs and lastly Tregs can generate from helper T cells in the TME [25]. All these in the long run lead to the selective buildup of immunosuppressive Tregs in the TME and subsequent tumor development.

TREG CELLS EXPRESS SPECIFIC CHEMOKINE RECEPTORS FOR TUMOR-INFILTRATION

Phagocytic leukocytes experience robust and directed movements in response to chemo-attractant gradients, a characteristic feature that facilitates them to act as the first line of cell-mediated immunity against infection. Chemokines are chemotactic cytokines that coordinate the movement and positioning of leukocytes [26]. Locomotion of immune cells are coordinated and guided by the temporal and spatial expression pattern of chemokines. Differential chemokine receptors expression on the leukocytes surface results in selective distribution of leukocytes under precise circumstances in different cellular compartments. The communication of chemo-attractants with leukocytes initiates a sequence of synchronized biochemical and cellular events that includes changes in integrin avidity and transmembrane potential, ion fluxes, changes in cell shape and production of superoxide anions [27]. These functions ultimately lead to the enhanced locomotion of the leukocytes. The chemokine superfamily involves roughly 50 chemokine ligands and 20 G protein-coupled seven-transmembrane signaling receptors. Chemokine ligands are divided into four subfamilies (CC, CXC, CX3C and XC) based on the position of the first two N-terminal cysteine residues. Chemokine receptors are part of a larger superfamily of G-protein-coupled receptors that include receptors for neurotransmitters, hormones, inflammatory substances,

proteinases, odorant and taste molecules, and even photons and calcium ions. Some receptors bind with CXC chemokines (CXCR1 to CXCR5), whereas others bind with the CC receptor family (CCR1 to CCR9) [27,28].

This infiltration of leukocytes is multifaceted in nature and consists of both pro-tumorigenic and anti-tumorigenic activities. The role of chemokines and chemokine receptors has been extensively studied in different human cancers. Literatures suggest that tumor cells, tumor associated cells and macrophages release certain chemokines that favor tumor growth by inviting immune suppressive cells in the tumor milieu which ultimately determine the course of cancer progression. Chemokines create a concentration gradient and Tregs expressing specific chemokine receptors respond by migrating towards high concentration [29]. Recent scientific discoveries suggest that Treg compartmentalization and migration is tissue specific. For selective retention of Tregs at specific locations distinctive chemokine receptor expression is required [30]. When Tregs move to sites of inflammation they express CCR2 and interact with CCL2 [31]. Likewise, for trafficking towards lymph nodes Tregs require the interaction of CCR7-CCL19 [32]. On the contrary, Tregs express a variety of chemokine receptors (CCR2-9, CXCR3/4) and infiltrate in response to tumor-secreted chemokines (CCL17, CCL22, CCL1, CCL28, and CCL9-11) in tumor condition [33]. A high-infiltration of Treg cells in TME is accompanied with poor prognosis, so tactics to control Treg infiltration are widely investigated

these days. It is also noted that the major interaction is facilitated by CCR4-CCL17/22, CCR10-CCL28, CCR8-CCL1, and CXCR3-CCL9/10/11 axes [34] (Figure 2).

SELECTIVE INFILTRATION OF T-REGULATORY CELLS IN THE TUMOR MICROENVIRONMENT BY THE INTERACTION OF CHEMOKINE RECEPTORS AND TUMOR-SECRETED CHEMOKINES

Up surged levels of Tregs has been seen in different human malignancies. The buildup of Tregs in TME suggests that Tregs are preferentially recruited and infiltrated within the TME. The mechanism of Tregs infiltration likely involves the interaction between chemokine receptors and tumor-secreted chemokines (Table 1).

Table 1: List of tumor-derived chemokines and Treg chemokine receptors that are involved in the tumor-infiltration: The major axes involved in this process are CCR4-CCL17/22, CCR5-CCL5, CCR6-CCL20, CCR8-CCL1, CXCR3-CCL9/10/11, CCR10-CCL28 and CXCR4-CXCL12.

Chemokine receptor on Treg	Tumor-derived chemokines
CCR4	CCL22/17
CCR5	CCL5
CCR6	CCL20
CCR8	CCL1
CXCR3/CCR5	CXCL9/10/11
CCR10	CCL28
CXCR4	CXCL12

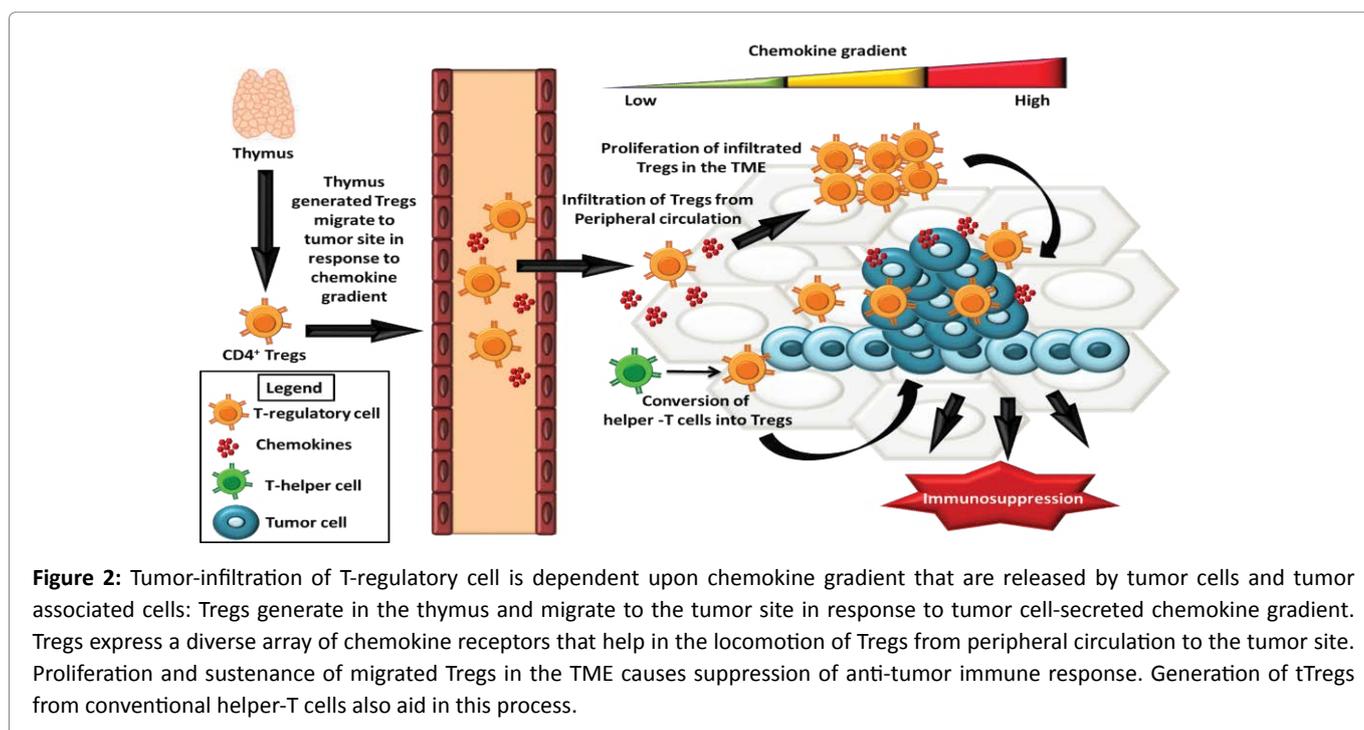


Figure 2: Tumor-infiltration of T-regulatory cell is dependent upon chemokine gradient that are released by tumor cells and tumor associated cells: Tregs generate in the thymus and migrate to the tumor site in response to tumor cell-secreted chemokine gradient. Tregs express a diverse array of chemokine receptors that help in the locomotion of Tregs from peripheral circulation to the tumor site. Proliferation and sustenance of migrated Tregs in the TME causes suppression of anti-tumor immune response. Generation of tTregs from conventional helper-T cells also aid in this process.

The CCL17/22-CCR4 axis is obligatory for recruitment of Treg cells into lung, lymphomas, ovarian, breast, gastric and prostate cancers (35,36). In case of ovarian cancer, cancer cells and cancer-associated macrophages release CCL22 and invites CCR4⁺ Treg cells in tumor microenvironment from lymph nodes resulting in destruction of anticancer immunity [37]. Augmented levels of CCL17 and CCL22 are known as poor prognostic markers in breast cancer. The same has been reported in cerebral spinal fluid of patients with lymphomatous and carcinomatous meningitis [38], gastric [39], and esophageal squamous cell carcinomas [40] because it causes infiltration of immunosuppressive CCR4⁺ Tregs. CCR4 is known to accumulate Treg cells in mouse melanoma model. Therefore, tumor environmental CCL22 facilitates Treg-cell tumor-infiltration. On the other hand, tumors that express negligible amount of CCL22 are not infiltrated by Tregs, suggesting that Treg recruitment to the tumor occurs via the CCL22:CCR4 axis.

FOXP3-expressing pancreatic ductal adenocarcinoma (PDAC) cells secrete CCL5 and recruit CCR5⁺ FOXP3⁺ Treg cells in the tumor. CCR5 is expressed not only by Tregs but other Teff cells also express CCR5 during tumor homing. But the expression by is very rare in Teff cells compared to Tregs suggesting more recruitment of Tregs than Teff cells in PDAC [41]. Tumor-associated macrophages (TAMs) develop from conventional macrophages during tumor condition and they work side-by-side with Tregs to destroy anti-tumor immunity. TAMs and Tregs positively regulate the functions of each other and their suppressive activities are somewhat similar. Tregs triggers the generation of TAMs; on the other hand, TAMs release CCL20 which recruits CCR6⁺ Treg cells in colorectal cancer [42]. In non-small cell lung carcinoma inflammatory cytokine CCL20 attracts CCR6⁺ Tregs in the tumor milieu and subsequent immunosuppression ensues. Chemotherapeutic drug against CCL20 reduces this infiltration. CCL1 remain up-regulated in breast cancer and secreted by myeloid cells in TME. CCL1 is revealed to have intense role in the gathering of CCR8⁺ Tregs in breast tumor milieu. CCR8⁺ Tregs overpower anti-cancer immunity by promoting CD39-mediated ATP-adenosine metabolism and emission of IL10 and granzyme-B. High infiltration of CCR8⁺FOXP3⁺ Treg cells is associated with poor prediction and CCL1 may act as a therapeutic target in breast cancer [43]. CXCR3⁺FOXP3⁺ T cells recruitment in TME has been shown to be associated with human breast, ovarian, colorectal, and hepatocellular carcinomas [44]. On the other hand, sporadic human renal cell carcinomas have increased expression of CXCL9, CXCL10, and CXCL11 which attracts CXCR3⁺CCR5⁺ T cells.

Hypoxia and hypoxia-inducible factors (HIF1 α /HIF1 β) establishes angiogenesis in tumor microenvironment and induces the expression of CCL28 which promotes the infiltration of CCR10⁺ Treg cells [45]. This phenomenon has been seen in ovarian cancer and liver cancer. Infiltrated CCR10⁺ Tregs in-turn secrete vascular endothelial growth factor-A (VEGFA), promoting angiogenesis and tumor progression. The same has been observed in mice metastatic primary tumors where Tregs are recruited by CCL8/CCR5 signaling [46]. CCR5 is also responsible for recruitment of Treg cells in skin squamous cell carcinoma (SSC) and colorectal cancer (CRC) [47]. This has been proved by the finding that, in CCR5^{-/-} mice tumor growth is delayed. High CCR5⁺ Tregs are more immunosuppressive compared to low CCR5⁺ Tregs. Hypoxia and VEGFA also increase the expression of CXCL12 in patients with basal-like breast cancers, which causes the infiltration of CXCR4⁺FOXP3⁺ Tregs [48,49]. Reports suggest that accumulation of these CXCR4⁺FOXP3⁺ cells in breast tumor tissue has been associated with poor diagnosis which is associated with immune suppression and more aggressive nature of this breast tumor. Hence, we can sum up that hypoxia and angiogenesis drives the trafficking of CCR10/CXCR4/CCR5⁺ Treg cells.

INFILTRATION OF TREG CELLS MAKES THE TME TOLEROGENTIC IN NATURE

Avoiding immune-surveillance is the prime characteristics of tumor and Treg cells assist in this process. Infiltrated Tregs suppress the action of tumor-specific Teff cells, promote immune evasion and develop a tolerogenic TME, where the immune cells show tolerant behavior against growing tumor. This phenomenon has been seen in several human malignancies like breast, ovarian, gastric, colorectal, pancreatic cancer, head and neck cancer to name a few [50]. Both nTreg and tTreg contributes to tolerogenic and immunosuppressive activity [51-53]. There are several strategies, by which Tregs exert their suppressive activity in the tumor milieu which are discussed below (Figure 3).

Immunogenic tumor cells recruit effector cells like CD4⁺ T cells, CD8⁺ T cells, NK and NK T cells, anti-tumorigenic macrophages and mature dendritic cells in the TME. These cells promote tumor cell killing by secreting IFN γ , TNF α , perforin, granzyme. On the other hand, tumor cells that do not express tumor cell-specific antigens recruit tolerogenic cells like Tregs, tumor associated macrophages, tolerogenic dendritic cells, myeloid derived suppressor cells. These cells secrete anti-inflammatory cytokines and molecules like IL10, TGF β , Gangliosides, IDO, Galectins that create an immunosuppressive milieu

that endorse tumor growth. The equilibrium between the function of these two types of cells ultimately determines tumor progression. IL10 and TGFβ are two very robust immunosuppressive cytokines that are released by Tregs and promote the energy of effector T cells, NK, NKT cells and APCs [54,55]. Recent scientific discovery stated that FOXP3 acts as a co-transcription factor with STAT3 that up-regulates IL10 expression in iTregs [56]. Other than Tregs, DCs and TAMs also release TGFβ, which transform activated CD4⁺CD25⁺ T cells into FOXP3⁺ Treg cells.

Treg induces Teff cell, NK cells, cytotoxic T lymphocytes (CTLs) and dendritic cells (DCs) apoptosis by secreting TNFα, perforin, granzyme A and B, Fas ligand. Tregs inhibits the activation of dendritic cells, promotes tumor-associated M2 macrophage (TAMs) activity. Most of the suppressive activity of Tregs is dependent upon the master transcription factor FOXP3 and its associated protein partners. FOXP3 activates the expression of inhibitory immune checkpoint molecules, such as, cytotoxic T-lymphocyte associated protein 4 (CTLA4), T-cell immunoglobulin and mucin-domain containing-3 (TIM3/HAVCR2), lymphocyte activation gene-3 (LAG3), programmed-death 1 (PD1), inducible T-cell costimulator (ICOS), and glucocorticoid-induced TNFR

family related gene (GITR); and T cell activation markers, CD25 and CD69 [57-60]. The cumulative effect of all these proteins down-regulates immune response and prevents excessive T cell activation during the course of tumor proliferation.

Immune checkpoint molecule PD-1 belongs to the immunoglobulin CD28 family and is expressed by different cells, including activated T cells, B cells, monocytes, NK cells and DCs. PD-L1 is the ligand of PD-1 which belongs to the B7 family. In addition to T cells, B cells, Tregs, macrophages and DCs, PD-L1 is extensively expressed on tumor cells, which contribute to the tumor immune escape. FOXP3 expression and Treg immunosuppressivity is increased by PDL1. Furthermore, PD-L1 can transform naive CD4⁺ T cells to Tregs through the down-regulation of Akt, mTOR and ERK2 and the concurrent up-regulation of PTEN. Another immune checkpoint molecule CTLA4 regulates the CD28 pathway. By binding with CD80 and CD86 ligand on DCs, CTLA4 blocks the costimulatory protein CD28 and limits dendritic cell activation. CTLA4⁺ Treg cells also secrete Indoleamine 2,3-dioxygenase (IDO) that catalyzes tryptophan breakdown and provides decreased costimulatory signal to DCs. IDO also blocks IL6 expression required for conversion of Tregs into

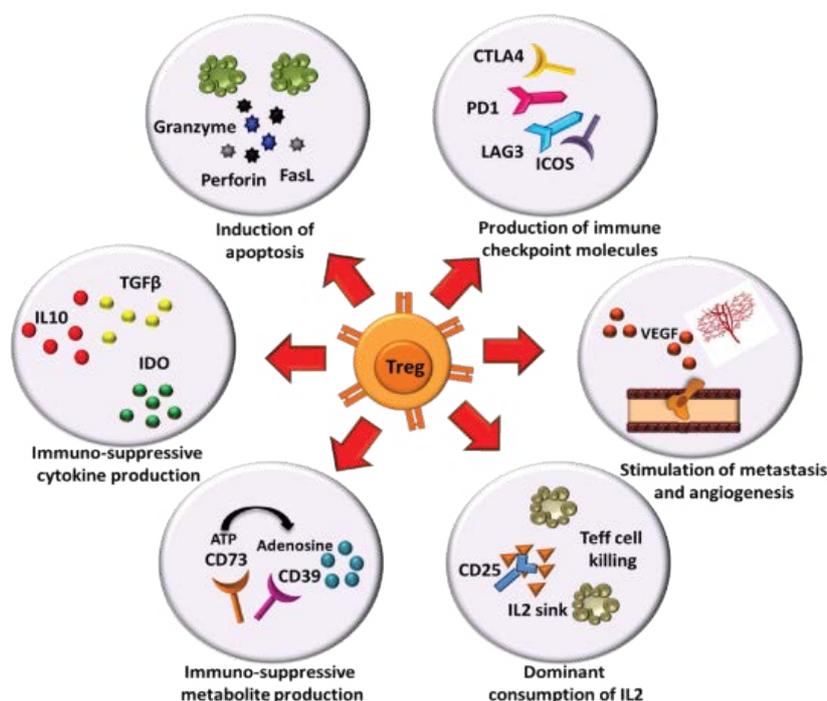


Figure 3: The manifold suppressive action of T-regulatory cells is indispensable for evasion of immune surveillance by growing tumor cells: Tregs induce apoptosis of Teff cells, produce immunosuppressive cytokines and metabolites which cause immune cell death. Presence of immune checkpoint molecules on the surface of Tregs is the primary cause for suppression of immune system. Tregs also promote metastasis and angiogenesis and dominantly consumes IL2 in the tumor microenvironment depriving other immune cells and causing their death. High Tregs infiltrated tumors are more invasive in nature and have poor prognosis.

Th17-like inflammatory Teff cells. LAG3, TIM3 and PD1 contribute to T cell cycle arrest, potent regulatory activity and generation of undeveloped antigen-presenting cells that are unable to elicit effector immune reactions against cancer [61,62]. LAG3 binds with MHC-II of DCs and prevents DC maturation and its effector function as antigen-presenting cell [63]. Tregs isolated from colorectal cancer (CRC) hepatocellular carcinoma (HCC) and pancreatic cancer patients show distinct expression pattern of CTLA4, ICOS, PD1, CD25 and CD69. Presence of immune checkpoint molecules on the surface of Tregs makes the immune system to secrete more anti-inflammatory cytokines and is the prime reason behind failure of immunotherapy.

Secretion and auto-uptake of tumor microenvironmental IL2 (through IL2R α /CD25 receptor) by Treg is an important constituent of Treg specific suppressive nature. IL2 controls FOXP3 expression by JAK-STAT pathway and subsequent expansion of Treg is inevitable. Tregs take up most of the IL2 present in the TME causing other Teff cells to be deprived of it and consequent BIM1-mediated Teff cell death [64-68]. Treg express ectonucleotidases CD39 and CD73 that hydrolyze exogenous ATP into AMP and immunosuppressive adenosine; they act together to confine CD80 and CD86 costimulatory signals of DCs and makes them nonfunctional. CD39 has been shown to be vastly expressed on infiltrating-Tregs in colon and head & neck cancers (HNC). Treg cells, NK cells and myeloid derived suppressor cells; these three potent suppressive cells create the immunosuppressive tumour network [69-72]. Treg cell infiltration leads to angiogenesis by secretion of VEGFA angiogenesis in ovarian cancer and liver cancer. Neuropilin-1 (Nrp1) derived from FOXP3+ Tregs acts on immature DCs and amends their functional activities. Latent TGF- β complexes which are anchored to the surface of T cells by glycoprotein-A repetitions predominant (GARP) can be cleaved to release active TGF- β in response. Latency-associated peptide (LAP) binds with TGF β in inactive latent TGF- β complexes and release active TGF β . Extremely immunosuppressive LAP+ and GARP/LAP co-expressing Tregs are present in excess amount in the TILs of CRC patients and the peripheral blood of pancreatic, CRC and their suppressive activity is facilitated by TGF β and IL10 [73-76].

It has been discovered that tumor-infiltrated Tregs show intensified suppressive activity and express Ki67, a potent proliferation marker when compared to Tregs isolated from peripheral circulation of tumor patients [77-80]. This happens owing to the heightened Treg activation within the TME, where Tregs are exposed to tumor-associated antigens.

CONCLUDING REMARKS

At the present time one of the emerging treatment strategies for treating cancer has been immunotherapy. Various attributes of immunotherapy are also being studied in pre-clinical and clinical trials. Some of these studies are showing promising results whereas in some patients there are instances of immunotherapy failure. Scientists around the world are trying to find the limitations of this failure. Several instances have shown that success and failure of immunotherapy largely depends upon the number and function of Treg cells. Suppressive and tolerogenic activity of Tregs in TME has gained clinical significance over the years. Different studies have focused on the ways of infiltration of Tregs in breast tumor and its subsequent consequences. Infiltrated Tregs acquire several mechanisms for the selective survival in the tumor-microenvironment which ultimately leads to the proliferation of Tregs in the vicinity of tumor. The balance between Treg and Teff cells disrupts which makes the TME more tolerogenic in nature. Recent advances in immunotherapy have focused on the blockade of the checkpoint molecules of Tregs or the adoptive transfer of (CAR-T) cells. Although these treatment strategies have generated considerable results in preclinical studies but not much success has been produced in clinical studies. TME plays a complex role in the survival of immunosuppressive Tregs which acts as a barrier for successful immunotherapy. Understanding the molecules involved in the infiltration of Tregs can be very beneficial in this context. Removal of inhibitory signal and immune checkpoint molecules are the key to the successful immunotherapy. Combining immunotherapy with traditional treatment strategies can also be used for selective depletion of Tregs which will help to reduce tumor volume.

DECLARATIONS

Ethical Approval and Consent to participate:

Not applicable.

Consent for publication:

All authors read and approved the final manuscript and concur with the submission for the publication.

Availability of supporting data:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

TS undertook the background literature study and prepared the initial draft of the manuscript and the figures; GS conceptualized, supervised the entire project and made final corrections to the draft.

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