J. Cell. Mol. Med. Vol 16, No 3, 2012 pp. 425-436

Regulatory T cell: a protection for tumour cells

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Received: June 02, 2011; Accepted: August 04, 2011

Abstract

Characterized by immunosuppression regulatory T cells (Tregs) play a key role in maintaining immune tolerance. A growing number of tumours have been found with Tregs accumulating in microenvironment and patients with high density of Tregs in tumour stroma get a worse prognosis, which suggests that Tregs may inhibit anti-tumour immunity in stroma, resulting in a poor prognosis. In this paper, we demonstrate the accumulation of Tregs in tumour stroma and the possible suppressive mechanisms. We also state the immunotherapy that has being used in animal and clinical trials.

Keywords: Tregs • TGF-β • IL-10 • Foxp3

Introduction

The concept of Tregs, called as suppressor T cells [1], was firstly mentioned by Gershon in 1970s. However the suppressor T cells were not successfully isolated for a lack of a reliable marker. Until 1995 Sakaguci *et al.* firstly reported that depleting $CD4^+CD25^+$ T lymphocytes by CD25 monoclonal antibody would induce multiple organs affected autoimmune diseases and reconstitution of those cells significantly prevented diseases development [2]. Since then $CD4^+CD25^+$ T lymphocytes have been described as Tregs and soon afterwards the discovery of transcription factor forkhead box P3 (Foxp3) provided us a better marker for identification of Tregs [3]. There are two major subsets of Tregs, naturally occurring regulatory T cells and antigen-induced regulatory T cells. Recently, another type of regulatory T cells originated from $CD8^+$ T cells have been reported by Suzuki [4]. But the function of these cells is still controversial.

Owing to the immunosuppression more and more autoimmune diseases and chronic inflammation have been found correlating to the disfunction or decreasing of Tregs *in vivo*, such as inflammatory bowel disease [5], systemic lupus erythematosus [6], multiple sclerosis (MS) [7], viral hepatitis type B [8] and so on. Secreting immunosuppressive cytokines and cell-to-cell connection has been generally acknowledged as the major manner to mediate immune tolerance. Tregs not only take part in the developing of autoimmune diseases, but also influence the prognosis of patients with cancer. Many tumours have been found with an increasing Tregs in tumour stroma, for example, breast cancer [9], hepatocellular carcinoma

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doi:10.1111/j.1582-4934.2011.01437.x

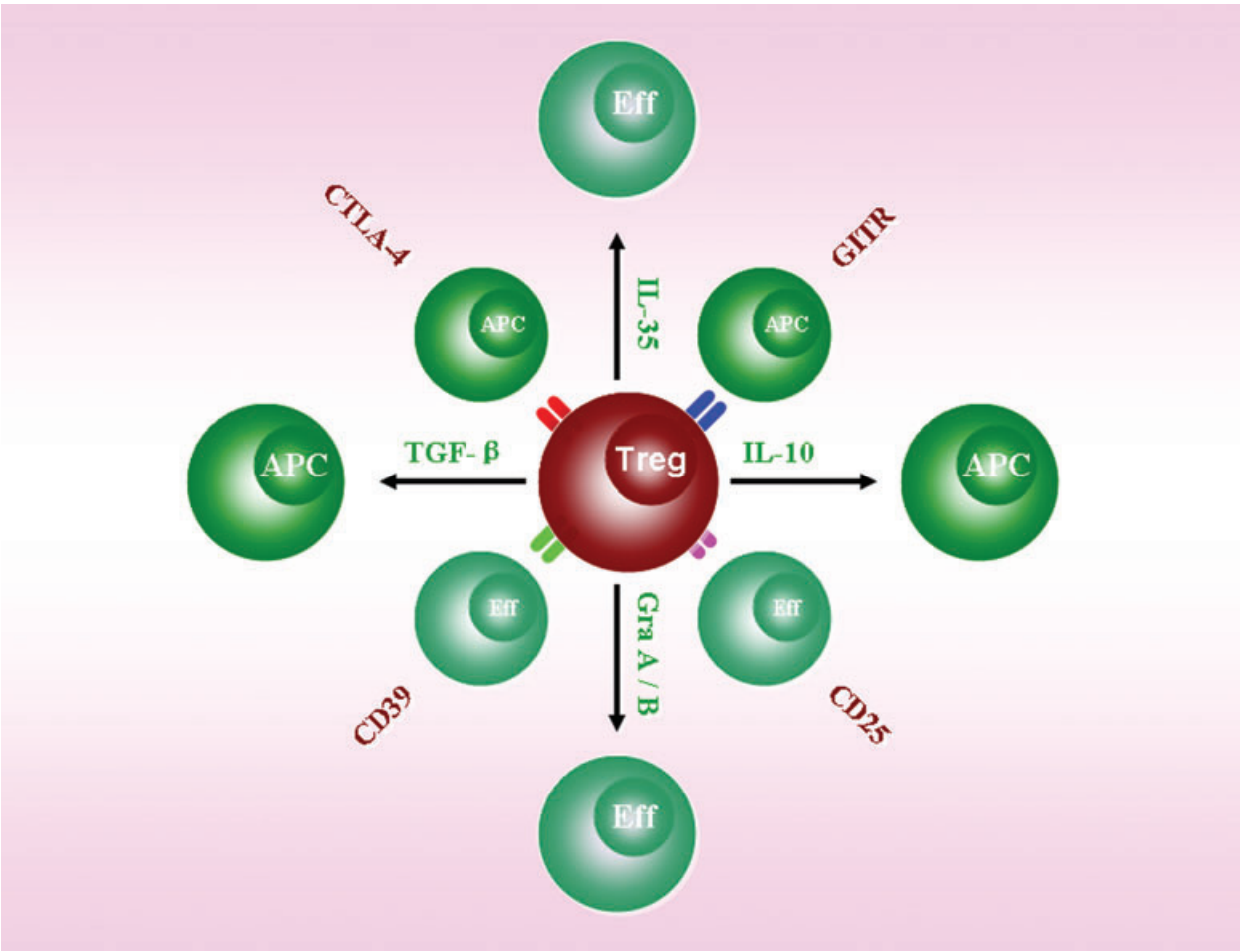

Fig. 1 Inhibitory cytokines and receptors used by regulatory T cells. Not just one mechanism participates in the process of suppression. Through secreting inhibitory cytokines (such as IL-10, IL-35 and TGF-β) regulatory T cells directly suppress effector T cells and APCs. Granzyme A/B dependent cytotoxicity mediate apoptosis of autologous targets cells, including effector T cells and DCs. CTLA-4 and GITR stop the activating signal transfer from APCs to effector T cells. Because of the higher affinity of CD25 to IL-2, regulatory T cells compete with effector T cells and induce the apoptosis of those cells.

[10], colon cancer [11, 12] and so on. The immunosuppression of Tregs to natural killer (NK) cells, $CD8^+$ T cells and antigen presenting cells has been considered as the major manner which helps tumour cells escaping from immune surveillance. More recently Tregs obtain unprecedented attention in the world; more and more scientists and doctors are intrigued in this field. In this paper, we summarize recent data which focus on Tregs trafficking, immunosuppression and treatment in patients with tumours.

The mechanisms of Tregs stimulating metastasis and mediating immunosuppression

Tregs are essential to self-tolerance and homeostasis, and they can even stimulate metastasis directly [13]. Tregs perform immunosuppression in tumour *via* connection and non-connection manners. Suppressive cytokines produced by Tregs such as transforming growth factor-β (TGF-β) and Interleukin-10 (IL-10) directly suppress immune responses [14–16]. However, the cell surface ligands cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) and glucocorticoid-induced tumour-necrosis factor-receptor related protein (GITR) can also mediate immunosuppression [17, 18] (Fig. 1).

Tregs stimulate cancer metastasis through RANKL-RANK signal

As mentioned earlier, an enhanced frequency of Tregs were found in peripheral blood and tumour stroma of several tumours. It has been controversial for a long period of time whether Tregs could promote tumour progression directly. But the recent studies have

confirmed that Tregs can mediate metastasis by receptor activator of nuclear factor-κB ligand (RANKL)-RANK signal [13]. Maspin is the unique member of serpin family characterized by inhibiting tumour angiogenesis. Zhang *et al.* reported that transferring Maspin gene into human prostate tumour could effectively inhibit tumour growth in mice and reduce the tumour microvessel [19]. However, tumour infiltrating cells expressed RANKL, which inhibited Maspin transcription and promote cancer metastasis [20]. More recently, Tan *et al.* have shown that Tregs were the major source of RANKL and stimulated pulmonary metastasis of human breast cancer. Blocking RANK signalling might prevent the recurrence of metastasis after surgical operation [13]. As a potential immunotherapeutic target RANK–RANKL signal pathway should merit further investigation.

TGF-β inhibits anti-tumour immunity in tumour microenvironment

Transforming growth factor-β is an essential for Tregs-mediated immune tolerance. In tumour microenvironment including Tregs, tumour cells, macrophages, endothelial, mesenchymal cells and myeloid precursor cells are the major sources of TGF-β [21]. The major function of TGF-β is to maintain self-tolerance and inhibit immune responses [22]. Nearly all of haemocytes are influenced by TGF-β *in vivo*, for example, T lymphocytes, B lymphocytes, NKs, dendritic cells (DCs), macrophages. Transforming growth factor-β could inhibit proliferation, differentiation and maturation of T lymphocytes or B lymphocytes [22, 23]. NKs play a key role in anti-tumour immunity by lysis, but Tregs can also suppress their cytotoxicity to tumour cells by TGF-β manner [24]. Antigen presenting cells are essential for activating body anti-tumour immunity through expressing co-stimulatory molecules. However, Tregs inhibit DCs and macrophages expressing co-stimulatory molecules by secreting TGF-β, and attenuate body anti-tumour immunity [25, 26]. But Nakamura *et al.* reported that surface binding TGF-β of Tregs but not secreting TGF-β mediated the suppression [27].

Immunosuppression of Tregs can be abolished by neutralized or knocked out IL-10

Interleukin-10 is another immunosuppressive cytokine secreted by Tregs [16]. Binding to receptor on membrane surface IL-10 transfer signal into cytoplasm and phosphorylate signal transducers and activators of transduction 3 (STAT3). Signal transducers and activators of transduction heterodimers subsequently transfer into nucleus and interact with IL-10 responsive gene [28]. Animal experiment has proved that transferring Tregs from wild-type mice but not from IL-10 deficient mice can resolve establishment colitis [29]. Interleukin-10 not only participates in the process of autoimmune diseases, but also weakens immune surveillance. In tumour models knocking out IL-10 gene or blocking IL-10 receptor dramatically activate $CD8^+$ T cells–mediated anti-tumour responses *in vivo* [30]. Similarly, in patients with head and neck squamous cell carcinoma Tregs isolated from tumour-mediated stronger suppression than those from healthy people [31]. Moreover, neutralizing IL-10 completely abrogated suppression of those cells *in vitro* [31]. Undoubtedly, IL-10 is a very important suppressor factor secreted by Tregs.

CTLA-4 participates in the suppression of Tregs

Cytotoxic T lymphocyte–associated antigen 4 is constitutively expressed on $CD4^+CD25^+$ regulatory T cells and plays a pivotal role in T lymphocytes–mediated immune responses [18]. Blocking CTLA-4 will induce chronic organ specific autoimmune diseases such as inflammatory bowel disease, diabetes and so on [32, 33]. The activation of T lymphocytes is a complex procedure, which needs stimuli from major histocompatibility complex (MHC) and B7 family members. Only when T cell receptor (TCR) and CD28 are triggered could T lymphocyte be fully activated. However, CTLA-4, the homologue of CD28, competitively binds to B7 family members and blocks the interaction between CD28 and B7. In contrast to CD28 pathway, CTLA-4 transfers a negative signal into peripheral T cells and decreases the production of IL-2, which is a very important cytokine to stimulate T cells proliferation [34]. In the absence of CTLA-4 peripheral T cells show a marked proliferation and secretion. Furthermore, most cell cycle progressions are prolonged and keep in the S and G_2-M phase [35]. Animal experiment has also proved that knocking out mouse CTLA-4 gene would induce sever multiple organ lymphocytic infiltration and die in 3–4 weeks [36]. However, transplanting bone marrow from wide-type mouse into CTLA-4-deficient mouse displayed an amazing result as multiple organs inflammation disappeared and the mouse's lifetimes were prolonged [37]. Cytotoxic T lymphocyte–associated antigen 4 is an indispensable inhibitory receptor for Tregs. Blocking CTLA-4 is an important manner to break immune tolerance and many experiments have shown that it is efficacy to treat cancer patients [38, 39].

Blocking GITR weakens the immunosuppression of Tregs

Glucocorticoid-induced tumour-necrosis factor-receptor related protein is a family member of tumour necrosis factor–nerve growth factor (TNF–NGF) receptor and predominantly expressed on $CD4^+CD25^+$ regulatory T cells [17]. It has been proved that blocking GITR *in vivo* will induce severe autoimmune diseases of model mouse [40, 41]. Administration of anti-GITR monoclonal antibody to tumour-bearing mouse could provoke anti-tumour immunity and lead to the regression of tumours. Furthermore, there were more infiltrating $CD4^+$ and $CD8^+$ T cells in treated tumours than control [42]. Because in some conditions GITR could induce T cells proliferation [43], the mechanism of

GITR-mediating negative regulation is still not clear. One possible explanation is that GITR located on the membrane of Tregs has a higher affinity to bind with GITRL, which competitive inhibit GITRL connecting with GITR on effector T cells.

Others

With the advent of researching, more and more mechanisms have been found to relating to the negative regulation of Tregs. For example, IL-35 is a newly discovered cytokine, which is characterized by negative regulation. It is one of the family members of IL-2 and a heterodimer consisted with IL-27β and IL-12β subunits [44, 45]. As a newly found inhibitory cytokine IL-35 plays a principal role in keeping immune homeostasis. It can suppress proliferation of conventional T cells collaborating with IL-10 [44]. Tregs isolated from IL-35 knockout mouse had a significantly reduced negative regulation and failed to control inflammatory bowel disease [45]. Moreover, recent studies have confirmed that IL-35 is another one important cytokine-mediated immunosuppression in tumour microenvironment [46].

CD39, an ectoenzyme binding on membrane of Tregs, can mediate immune suppression by catalysing adenosine generation. Tregs isolated from CD39 knockout mouse have an impaired negative regulation [47]. Compared with mouse, CD39 is restricted to a subset of $Foxp3^+$ regulatory T cells and decreases significantly in patients with relapsing form of MS [48]. The decreasing of CD39 may be the major cause leading to impair suppressive capacity of Tregs. In addition, apoptosis induced by perforin/granzyme A and B manner is another way to mediate suppression by regulatory T cells in human and murine [49, 50]. Tregs consumed abundant IL-2 in microenvironment *via* CD25 and induce cytokine deprivation mediated apoptosis of effector T cells [51].

The manners of Tregs in tumour stroma

A growing number of tumours, such as hepatocellular carcinoma [10], colon carcinoma [12], ovarian cancer [52] and breast cancer [9], have been found to have a microenvironment with high frequency of Foxp3-positive cells. Moreover, patients with high Tregs usually have a poor survival than the lower ones. Proliferation, recruitment and conversion are considered to the major ways that contribute to the accumulation of Tregs in tumours (Fig. 2).

Tregs proliferate in tumour microenvironment

In tumour microenvironment, Tregs play a key role in suppressing effector T cells. However, a subset of myeloid DCs in tumours could be converted into regulatory DCs characterized by secreting bioactive TGF-β and mediated proliferation of naturally occurring Tregs [53]. Coe *et al.* reported that tumour antigen contributed to the expansion of natural regulatory T cells in tumour and $CD11c^+$ cells purified from tumour-draining lymph nodes were the key cells involved in the antigen-presenting process. Moreover, TGF-β expressed by tumour cells was also responsible for the expansion of Tregs [54]. On the one hand, expansion induces a high density of Tregs in tumour stroma; on the other hand, the increasing Tregs abrogate anti-tumour immunity *in vivo*, resulting in a poor prognosis. Therefore, blocking the proliferation might improve the survival of patients with tumour.

Tumours secrete chemokines recruiting Tregs from peripheral blood

Chemokine is a big family, which mediates lymphocyte and granulocyte migrating from peripheral blood into damaged tissues, especially in inflammatory condition. Plenty of tumours are found to secrete chemokines such as stromal cell-derived factor-1 (SDF-1/CXCL12), thymus and activation-regulated chemokine (TARC/CCL17), macrophage-derived chemokine (MDC/CCL22) and so on [55, 56]. In tumour stroma, chemokines not only regulate neoplasm metastasis, but also mediate Tregs homing [57]. Tregs functionally express CCR4, the unique receptor of CCL22, and recruit from peripheral blood into solid tumours [58]. Through the interaction Tregs are trafficked and activated in tumour stroma [59]. Moreover, Mizukami reported that the migration could be significantly abrogated by CCL22 neutralizing antibody [56]. It proves from negative side that chemokine really contribute to Tregs homing.

Non-regulatory T cells converse into Tregs in tumour stroma

Accumulating evidences have shown that Tregs can be conversed from non-regulatory T cells *in vivo* and *in vitro* [60–63]. Conversion is another important reason to induce increasing frequency of Tregs in tumour microenvironment. Several kinds of cytokines and immune cells join in the process, such as TGF-β, IL-2, retinoic acid (RA), DCs and macrophage [62–64]. As a considerable negative regulator TGF-β is the first cytokine confirmed with Foxp3 inducible function [60]. In microenvironment tumour cells could interact with APCs and enhance the secretion of TGF-β. Under the action of TGF-β, $CD4^+Foxp3^-$ T cells are conversed into $CD4^+Foxp3^+$ regulatory T cells. Nevertheless, neutralizing antibody thoroughly could abrogate the inducible function of TGF-β [65].

Retinoic acid is a newly found factor, which could induce the expression of Foxp3 gene in $CD4^+Foxp3^-$ T cells, and synthetic RA receptor inhibitors block the expression of Foxp3 gene, suggesting that RA, the metabolin of vitamin A, is essential for the conversion of Tregs *in vitro* [61]. In addition, macrophage is also related to the increased intratumoural Tregs *via* serial section authors found that patients with high intratumoural macrophage density tended to

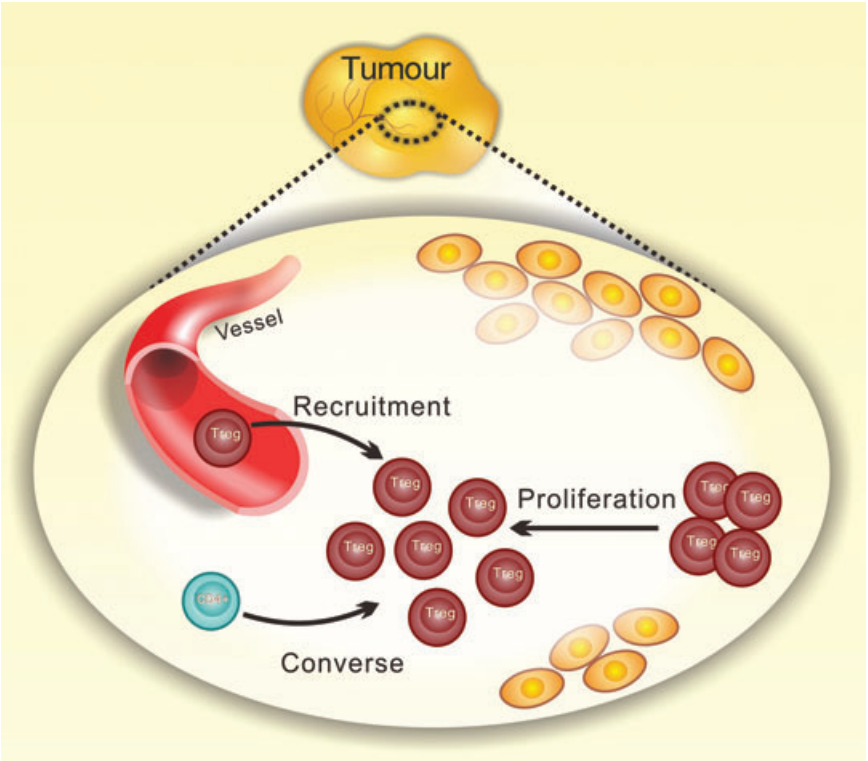

Fig. 2 The manners of the accumulation of regulatory T cells in tumour. There are three manners of multiplying Tregs in tumour: (1) malignant cells secreting chemokines and recruiting Tregs infiltrating into stroma from peripheral blood, (2) TGF-β secreted by tumour cells and others conversing $CD4^+$ non-regulatory T cells into Tregs and (3) Tregs in tumour stroma proliferated by the stimulation of cytokines and APCs.

have more Tregs than those with low macrophage density and depletion of macrophage effective decreased the density of Foxp3 positive cells in tumour stroma [63]. But the exact link between macrophage and Tregs is still elusive.

The target cells of Tregs in tumour microenvironment

The major roles Tregs plays in *in vivo* are keeping homeostasis and inhibiting severe immune responses. Most of immune cells in the tumour stroma, such as T lymphocytes, B lymphocytes, NK, DCs, macrophage, are targets of Tregs [66–71]. Redundant Tregs accumulating in a tumour not only keep homeostasis, but also restrain local anti-tumour immunity (Fig. 3). Because of the suppression, tumour cells evade from body immune surveillance, hence a poor prognosis.

Tregs weaken body immune surveillance by inhibiting DCs

Dendritic cells are the most considerable professional antigen presenting cells *in vivo* and play a critical role in defence of infection and tumour. There are two major subsets of DCs widely distributed in organs and lymphatic tissues, myeloid DCs and lymphoid DCs [72]. In tumour microenvironment, DCs migrate from tumour into lymph nodes and mature with the tumour antigen stimuli. In this process, DCs capture antigen and form peptide–MHC complexes on cell surface. Meanwhile, the expression of co-stimulatory and adhesion molecules is also enhanced. Major histocompatibility complex complexes interact with TCR and provide the first signal for T cell activation. Co-stimulatory molecules that bind to respective receptors on T cells, including CD80, CD83, CD86, provide the second signal to activate T cells. Both of the two signals are needed in stimulating T cells proliferation and activation.

But DCs are an important target of Tregs in tumour stroma. Inhibiting the expression of co-stimulatory molecules is one of most important ways to suppress DCs. Most of co-stimulatory molecules expressing on DCs surface, such as CD80, CD83, CD86 and so on, significantly low regulate during co-culture with Tregs [67, 69]. Lack of stimulation from co-stimulatory molecules T cells can not be activated sufficiently and body immune surveillance is significantly impaired. Although TGF-β and IL-10 secreted by Tregs mediate the suppression, cell-to-cell connection should not be ignored [67]. Previously, several clinical trials were conducted to treat patients with cancer by DCs vaccines, but no firm conclusions were yielded [73]. Therefore, Tregs can be one of the key contributing factors to weak immunotherapy of DCs vaccines *in vivo*, which merits further investigation.

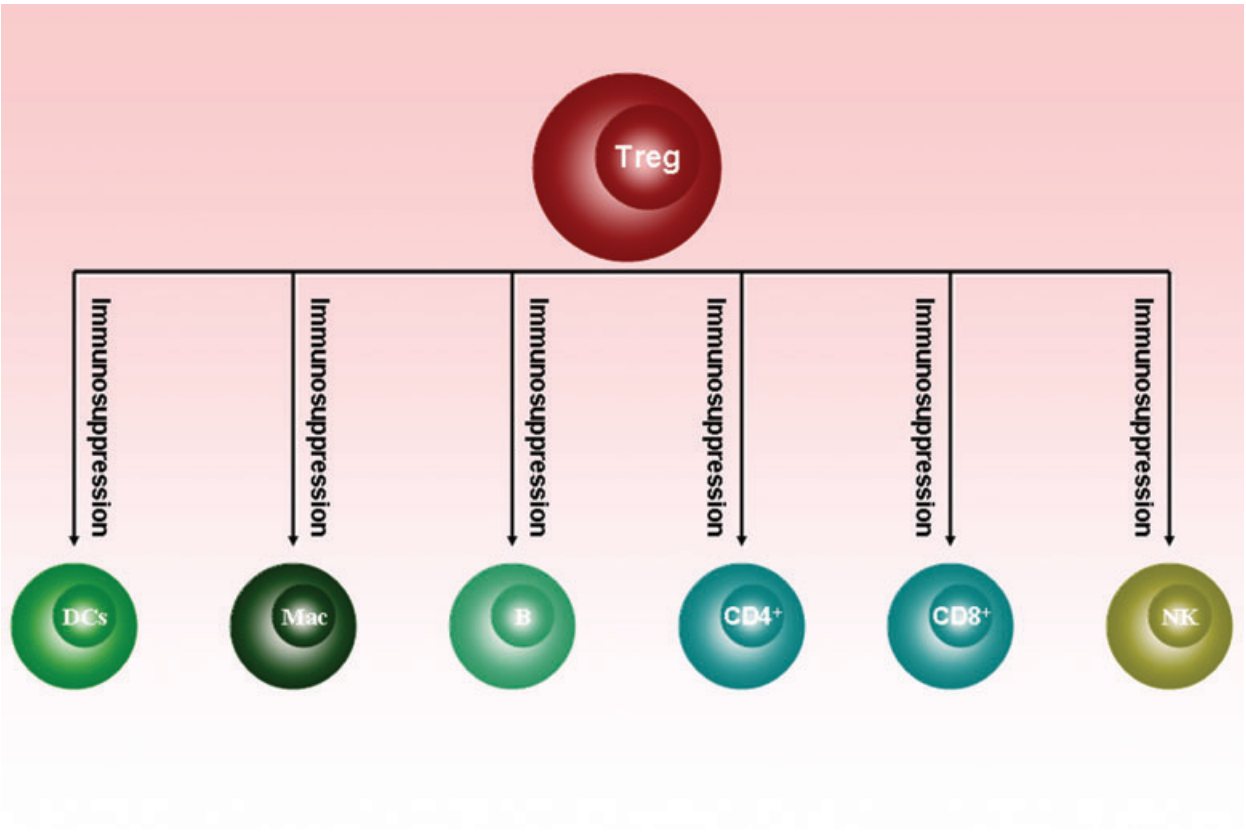

Fig. 3 The target cells of regulatory T cells. By secreting inhibitory cytokines and expressing suppressive receptor, regulatory T cells not only keep homeostasis *in vivo*, but also inhibit immune surveillance in tumour microenvironment. Regulatory T cells nearly suppress all of lymphocytes, including T cells, B cells, NKs, DCs, and macrophage (Mac). Because of the suppression, anti-tumour immunity is significantly weakened.

Indoleamine 2,3-dioxygenase (IDO) expressed by DCs is capable of suppressing proliferation of T cells *in vitro*. *Via* engagement by CTLA-4 situate on Tregs the expressing of IDO could be trigged [74]. In addition, IDO expressing mature monocyte-derived DCs expanded autologous Tregs [75]. Even in suitable conditions DCs could promote the conversion of naive T cells into Foxp3$^+$ regulatory T cells [61]. It becomes an infernal circle and weakens the antigen presenting of DCs.

Tregs suppress non-regulatory CD4$^+$ T cells

T cells–mediated immune responses is an important part of anti-tumour immunity *in vivo*. Non-regulatory CD4$^+$ T cells are a major subset of peripheral blood T cells and play an important supporting role in tumour immunity. After peptide–MHC II complexes and co-stimulatory molecules double stimulating, those cells are activated and secrete plenty of cytokines such as IL-2, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and so on. CD4$^+$ T cells mediate the function of CD8$^+$ T cells, NKs and DCs *via* releasing of cytokines and cell-to-cell connection [76–78]. Although Tregs are also a part of CD4$^+$ T cells, they have completely different function *in vivo* or *in vitro*. Tregs, especially infiltrating into tumour stroma, significantly inhibit CD4$^+$CD25$^-$ effector T cells activation and releasing cytokines, which suppress tumour immunity directly [79, 80]. During the suppression CTLA-4, IL-10, TGF-β and other factors play a vital important role [16, 22]. In addition, CD4$^+$Foxp3$^-$ T cells can be conversed into CD4$^+$Foxp3$^+$ regulatory T cells, which is another significant manner to help tumour cells escape from immune surveillance.

Tregs inhibit the proliferation and anti-tumour capacity of CD8$^+$ T cells

CD8$^+$ T cells, characterized by anti-tumour, are the most important source of CTL *in vivo*. MHC-I and B7 are two necessary signals to activate CD8$^+$ T cells. After stimulating and interacting with CD4$^+$ T cells, CD8$^+$ T cells converse into CTL. CD8$^+$ CTL-mediated tumour cell lysis include three major manners: granule exocytosis, Fas/Fas ligand (FasL) death pathway and releasing cytokines [81–83]. Through granule exocytosis, perforin and

granzymes are delivered to tumour cells and lead to lysis [84]. In addition, interaction with Fas expressed on target cells $CD8^+$ CTL directly mediates the target cell death. Otherwise TNF-α released by $CD8^+$ CTL is also an important manner to kill malignant cells [81]. Through inhibiting $CD8^+$ T cells proliferate and infiltrate into tumour microenvironment, Tregs severely impaired the anti-tumour capacity of $CD8^+$ T cells [66, 85]. So it was not surprising that depletion of tumour-associated regulatory T cells improved the efficacy of adoptive transfer CTL in murine acute myeloid leukaemia [86].

Tregs suppress the lysis of NK cells

Although NKs possess little frequency of peripheral lymphocytes, it plays a key role in anti-tumour immunity. Natural cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC) and cytokine production are considered the principle manners which mediate the lysis of NKs. MHC-I molecules transmit an inhibitory signal to NKs *via* killer cell inhibitory receptors (KIR). Moreover, killer cell activating receptors (KAR) expressed on NKs surface transmit an opposite signal. The balance between inhibitory and activating signals determines the cytotoxicity of NKs. In contrast to normal cells, some tumour cells generally low express MHC-I molecules, which break the balance and activate the lysis of NKs [24].

Similar to T cells, NKs are also sensitive to the immune suppression of Tregs. Tregs not only inhibit proliferation of NKs, but also suppress cytokines production and activation [70, 87, 88]. *Via* expressing IFN-γ and perforin NKs mediate the target cell lysis. However, co-culture with Tregs significantly decreased the expression of the both molecules [70]. In addition, natural-killer group (NKG)-2D is a crucial KAR resided on membrane of NKs and plays a pivotal role in anti-tumour immunity. But Tregs could low regulate the expression of NKG-2D by membrane binding TGF-β [87]. Because of the immune suppression of Tregs, lysis of NKs is significantly impaired and helps tumour cells escape from immune surveillance.

Tregs inhibit the proliferation of B cells

B lymphocytes are another kind of APCs *in vivo* and also express co-stimulatory molecules. As in the case of DCs, B cells are effective antigen presenting cells and mediate tumour immunity *in vitro* [89]. However, only activated B cells could effectively stimulate $CD4^+$ T cells, resting B cells even block anti-tumour responses *in vivo* [90, 91]. Tregs can also suppress the function of B cells and influence tumour immunity mediated by B cells. Shevch *et al.* reported that Tregs directly suppressed B cells proliferation and increased the death of the cells by cell-to-cell connection [71]. Otherwise, Tregs directly inhibited Ig production in B cells, which also demonstrated the suppressive relationship between Tregs and B cells [92].

Tregs inhibit function of macrophage directly

Although many studies showed that macrophage infiltrating in tumour produces a negative effect on prognosis [93, 94]. As an important member of professional APC, macrophage also participates in the body anti-tumour immunity. Macrophage potentially functions in anti-tumour immunity by directly killing cancer cells, presenting tumour-associated antigens and secreting cytokines [95]. Moreover, some results have shown that patients with high macrophage density in tumour get improved prognosis [96, 97]. But there is little literature on the relationship between Tregs and macrophage. Gerwenka *et al*. reported that Tregs depletion in mice led to tumour rejection and macrophage accumulation [98], suggesting that Tregs in tumour might inhibit macrophage migrating into tumour stroma. In addition, co-culture with Tregs induced minimal cytokine production and limited up-regulation of CD40, CD80 and CD86 in monocyte/macrophage [68]. Inextricably, there is a close link between macrophage and Tregs.

A new immunotherapeutic target

We have demonstrated a negative connection between Tregs and human cancer. Because of the immunosuppression Tregs have become another prospective therapeutic target. Many drugs and antibodies for depletion those cells have been designed and applied in clinical trials including cyclophosphamide, anti-CD25 antibody, anti-GITR antibody, denileukin diftitox and so on. Eliminating Tregs can inhibit tumour growth or even trigger tumour regression, especially in some animal models [99–102], and blocking Tregs may break immune tolerance and activate anti-tumour immunity, as indicated by some clinical favourable results [39, 103].

Depletion of Tregs leads to tumour regression

As a common agent, cyclophosphamide is mainly used for the treatment of malignant cancer and autoimmune diseases. It can effectively inhibit synthesis of DNA and induce apoptosis of target cells. Animal experiments show that cyclophosphamide can directly deplete Tregs and improve outcome of tumour-bearing animals [104]. Although animal experiments have achieved excited results, the outcomes of clinical trials are not consistent with each other [105, 106]. It is still controversial that whether cyclophosphamide can decrease the frequency of Tregs or improve prognosis in human patients. As a specific marker of Tregs, CD25 has become a therapeutic target for depletion of those cells. Several studies have indicated that administration of anti-CD25 mAb significantly prevented tumour growth or even got tumour regression in mouse [99–102]. Denileukin diftitox (ONTAK) is a novel recombinant fusion protein characterized by a fragment of diphtheria toxin linked to human interleukin-2 [107].

It was first used to treat cutaneous T cell lymphoma and acquired satisfactory responses [103]. Thanks to the significant depletion of Tregs in circulating pool, doctors tried it in treating patients with melanoma and obtained favourable results [108].

Although eliminating regulatory T cells have shown a potential immunotherapy for cancer treatment, the shortcomings are also obvious. Firstly, some effector cells can also express CD25, and administration of CD25 mAb may induce the depletion of CD25-positive effector T cells and impair anti-tumour immunity [109]. Secondly, Tregs elimination breaks self-tolerance and leads to autoimmune diseases [2]. Tregs are clearly an important target for immunotherapy, but not the best choice. The only effective solution depends on the discovery of more specific markers.

Inhibiting the immunosuppression of Tregs improves the prognosis of cancer patients

Glucocorticoid-induced tumour-necrosis factor-receptor related protein and Cytotoxic T lymphocyte–associated antigen 4 are the significant immunosuppressive receptors located on Tregs membrane. Because of the immunosuppression, those receptors have become a new target to attenuate the function of Tregs. Several studies have reported the potential efficiency to treat tumour with agonistic anti-GITR mAb (DTA-1) and obtain favourable outcomes [42, 110]. Administration of anti-GITR mAb not only attenuated Tregs-mediated immunosuppression, but also enhanced $CD4^+$ and $CD8^+$ T cells infiltrating into tumour [42]. As a negative regulatory receptor CTLA-4 blockage has been proved to be another valid manner to activate anti-tumour immunity [111, 112]. It could enhance the production of IFN-γ and stimuli the anti-tumour responses in early stages of tumour growth [112]. Clinical trials have demonstrated that receiving an i.v. infusion of anti-CTLA-4 mAb, Ipilimumab or tremelimumab, successfully enhanced anti-tumour immunity in patients with solid tumours [38, 39]. But adverse effects induced by anti-CTLA-4 mAb should not be ignored. Blocking those regulatory receptors efficiently weakens immunosuppression and breaks self-tolerance mediated by Tregs. Apparently, signal drug treatment is not enough for the best efficacy,

Combination immunotherapy may be the best choice

A variety of anti-tumour vaccines have undergone clinical trials for years, but there have been no convincing results. The discovery of Tregs can provide us with a new path to the exploration of the mechanisms. As previously mentioned, Tregs mediate immunosuppression in tumour microenvironment and impair the body's anti-tumour immunity. Therefore, Tregs depletion and inhibition can have great potential in enhancing the therapeutic effect of vaccine. Recently, several reports have confirmed the curative effect of combination of Tregs' depletion and tumour vaccine [113, 114], which suggested that depleting Tregs *in vivo* significantly attenuated Treg-mediated immunosuppressive activity and collaborated with tumour vaccine to evoke efficient anti-tumour responses [113, 114]. Moreover, animal experiments have shown that vaccines combination with CTLA-4 blockage therapy is capable of eliciting anti-tumour responses and inhibiting tumour growth too [115, 116]. In addition, the similar experiment reported that using anti-GITR antibody combination with a DNA vaccine leaded to a more effective therapy than vaccine alone [117]. To surprise us is that several clinical trials have also proved the synergistic effect of combination therapy [113, 118]. However, we should recognize that immunotherapy is just the beginning and needs further evidence-based investigation.

Conclusions

Tregs accumulating in tumour stroma have been found in a growing number of tumours. Although the fact that those cells are depleted in mice shows a great prospect for tumour treatment, it is still a question whether the same results will occur in humans. Only a small percentage of patients with tumour have acquired immune responses and improved outcomes, suggesting that the depletion of Tregs by CD25 mAb or blockage inhibitory receptor is not enough to abrogate the suppression of Tregs in tumour stroma. Therefore, blocking the migration or hamper the reverse of Tregs might become a new path in breaking the tolerance in tumour microenvironment. Unfortunately, we are not sure about the exact mechanism of how Tregs accumulate into tumours. Chemokine can partially explain the recruitment of Tregs into ovarian and gastric cancer, yet it still needs further investigation to confirm whether it can produce a marked effect in other solid tumours. Similarly, TGF-β is not the only reason to explain the conversion of Tregs in tumour stroma. Thus it is still need more work to do that how $CD4^+$ T cells converse into Tregs in tumour stroma. In spite of the difficulties we have met, we believe that with further investigation immunotherapy will become an important means of treating cancer, and that Tregs can be a good choice for immunotherapy!

Acknowledgements

The authors thank the team members of Prof. Shen, Xizhong's laboratory for their helpful discussion and critical reading of the manuscript. This work was partially supported by the Ministry of Health (2009ZX10004-301), China National Key Projects for Infectious Diseases (2008ZX10002-017) and China Postdoctoral Science Foundation funded project (20100480542).

Conflict of interest

The authors declare that there are no conflicts of interest.

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