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The opposing roles of $CD4^+$ T cells in anti-tumour immunity

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Summary

Cancer immunotherapy focuses mainly on anti-tumour activity of $CD8^+$ cytotoxic T lymphocytes (CTLs). CTLs can directly kill all tumour cell types, provided they carry recognizable antigens. However, $CD4^+$ T cells also play important roles in anti-tumour immunity. $CD4^+$ T cells can either suppress or promote the anti-tumour CTL response, either in secondary lymphoid organs or in the tumour. In this review, we highlight opposing mechanisms of conventional and regulatory T cells at both sites. We outline how current cancer immunotherapy strategies affect both subsets and how selective modulation of each subset is important to maximize the clinical response of cancer patients.

Keywords: cancer; CD4 cell; regulatory T cell; T cell; tumour immunology.

Introduction

$CD8^+$ cytotoxic T lymphocytes (CTLs) are a major force of adaptive immunity and a perfect weapon to combat cancer. They can specifically recognize intracellular alterations as peptides presented by major histocompatibility complex (MHC) class I on almost all body cells and efficiently mediate cytotoxicity. To ensure their beneficial role, they are controlled on multiple levels. Negative selection of self-reactive T cells in the thymus is a primary mechanism by which immunological self-tolerance is maintained. Nevertheless, self-reactive T cells are found in tissues and blood of healthy individuals that have apparently escaped from this selection process.[1] Such self-reactive T cells need to be controlled by additional mechanisms to avoid harmful autoimmune responses. For this purpose, peripheral tolerance mechanisms exist that rely on the non-activated state of dendritic cells (DCs) and the activity of specialized regulatory T (Treg) cells.[2] Surveillance against (non-virally associated) cancers relies on self-reactive T cells, as tumour cells harbour antigens derived from endogenous proteins. Furthermore, tumour cells may not exude any molecules that can activate DCs. For these reasons, peripheral tolerance may have to be broken to elicit a CTL response to cancer.[3] Furthermore, CTL responses rely for the optimization and maintenance of their functionality on other immune cells. At the centre of keeping the balance between harmful and beneficial CTL responses lie $CD4^+$ T cells.

$CD4^+$ T cells recognize antigen in the context of MHC class II, which is primarily found on immune cells. A key role of $CD4^+$ T cells is therefore to modulate the state and function of other immune cells. $CD4^+$ T cells represent a diverse cell population with many differentiation states that have all been implicated in controlling immune responses against cancer.[4] On one side of the spectrum are $CD4^+$ T conventional (Tconv) cells that promote anti-tumour immunity, either by direct elimination of MHC class II^+ tumour cells or indirectly through modulation of the tumour microenvironment.[4] Moreover, in secondary lymphoid organs, $CD4^+$ T cells improve the magnitude and quality of B-cell responses and CTL responses. On the other side of the spectrum are $CD4^+$ Treg cells that suppress CTL responses, either directly by production of inhibitory cytokines or indirectly by influencing state and function of DCs and other (innate) immune cell types.[5]

In this review, we describe the opposing roles of $CD4^+$ T cells in anti-tumour immunity. We outline the mechanisms by which Tconv and Treg cells can regulate the immune response at both the priming and the effector site, and how modulating these cell subsets can improve the efficacy of cancer immunotherapy.

The role of Tconv cells at the priming site

$CD4^+$ T cells can directly recognize antigen on tumour cells, in case these express MHC class II. However, Tconv cells mediate most of their immunomodulatory functions

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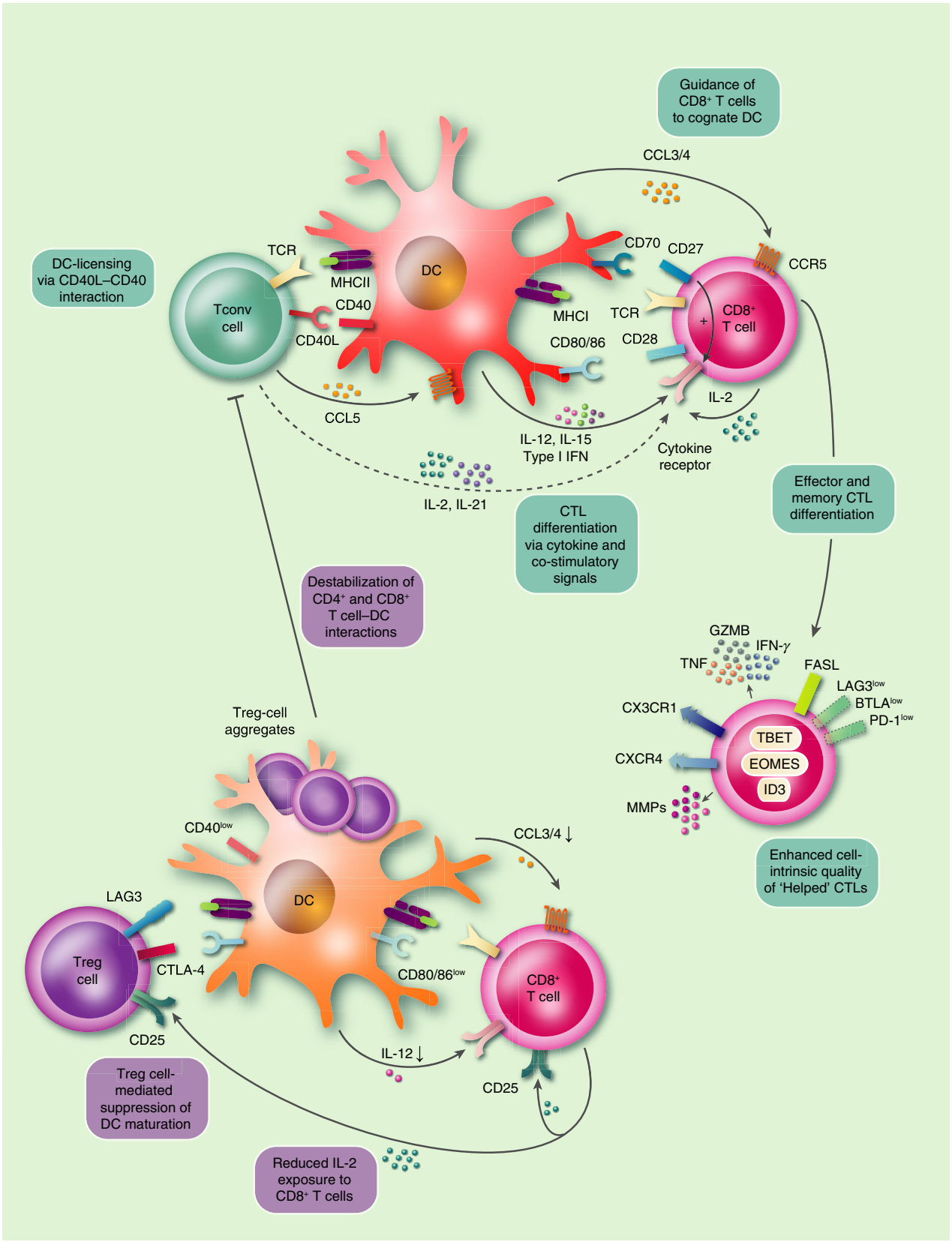
Guidance of CD8+ T cells to cognate DC
CCL3/4
DC-licensing via CD40L–CD40 interaction
TCR
MHCII
CD40
CD40L
Tconv cell
DC
CD70
CD27
CCR5
MHCI
TCR
CD8+ T cell
CD80/86
CD28
IL-2
CCL5
IL-12, IL-15 Type I IFN
Cytokine receptor
IL-2, IL-21
CTL differentiation via cytokine and co-stimulatory signals
Effector and memory CTL differentiation
Destabilization of CD4+ and CD8+ T cell–DC interactions
GZMB
IFN-γ
TNF
FASL
LAG3low
BTLAlow
PD-1low
CX3CR1
CXCR4
MMPs
TBET
EOMES
ID3
Enhanced cell-intrinsic quality of 'Helped' CTLs
Treg-cell aggregates
CD40low
CCL3/4 ↓
LAG3
Treg cell
CTLA-4
CD25
DC
CD80/86low
CD8+ T cell
IL-12 ↓
CD25
Treg cell-mediated suppression of DC maturation
Reduced IL-2 exposure to CD8+ T cells

Figure 1. The roles of conventional (Tconv) and regulatory (Treg) T cells in secondary lymphoid organs. Naive Tconv cells become activated and then interact and license XCR1$^+$ lymph node (LN) -resident dendritic cells (DCs) through a CD40-dependent process. Preactivated Tconv cells can recruit more DCs through secretion of CCL5. Licensed DCs attract preactivated CD8$^+$ T cells through the secretion of CCL3/4. Licensed DCs also express higher levels of co-stimulatory ligands CD70 and CD80/86 and secrete interleukin-12 (IL-12), IL-15 and type I interferon (IFN) to support effector and memory cytotoxic T lymphocyte (CTL) differentiation. Interaction with licensed DCs results in up-regulation of CD25 and IL-2 production by CD8$^+$ T cells. Tconv cells can also directly support the CTL response by secretion of IL-2 and IL-21. 'Helped' CTLs express high levels of transcription factors – T-bet, Eomes and Id3, effector molecules – granzyme B (GZMB), tumour necrosis factor (TNF) and IFN-γ, chemokine receptors – CXCR4 and CX3CR1, matrix metalloproteases (MMPs) and lower levels of co-inhibitory receptors lymphocyte activation gene 3 (LAG3), B and T lymphocyte attenuator (BTLA) and programmed cell death protein 1 (PD-1). Treg cells destabilize the interaction of Tconv and CD8$^+$ T cells with DCs by limiting chemokine secretion by DCs and forming aggregates on their surface. Treg cells down-regulate the expression of CD80/86 by DCs and limit the availability of MHC class II to and CD80/86 through binding via LAG3 and CTLA-4, respectively. Treg cells can also limit the expression of IL-12 and CD40 by DCs and compete for IL-2 available to CD8$^+$ T cells.

by recognition of antigen on specialized antigen-presenting cells such as DCs and macrophages (Fig. 1). In the 1980s it was acknowledged that CD4$^+$ T cells can provide help for CTL priming[6] and later, DCs were found to serve as a platform by which the effects of CD4$^+$ T-cell help are mediated.[7] Accumulated data in various experimental systems suggested that CD4$^+$ T-cell help is only required to elicit a primary CTL response when direct activation of DCs by pathogen- or danger-associated molecular patterns is limited.[6] This was later suggested to depend on the amount of type I interferon (IFN) produced by such antigen-presenting cells.[8] However, CD4$^+$ T-cell help was always required to induce and maintain functional memory CD8$^+$ T-cell responses, even when strong inflammatory stimuli were present.[6] In agreement with limiting innate stimuli, CTL responses against cancer often rely on the provision of CD4$^+$ T-cell help.[9–11] Moreover, effective therapeutic vaccination strategies against cancer have been recently shown to induce potent anti-tumour CD4$^+$ T-cell responses directed against mutated or non-mutated cancer antigens.[12–14]

A primary mechanism by which CD4$^+$ T cells 'help' to induce CD8$^+$ T-cell responses is by increasing antigen-presenting and co-stimulatory capacities of DCs. A key signal in this so-called DC 'licensing' is delivered by interaction between CD40 ligand (CD40L) on the cognate CD4$^+$ T cell and CD40 on the DC, which allows for functional maturation of the DC.[15] In a newly recognized scenario elucidated by intravital microscopy in mice, T-cell priming occurs in two steps: CD4$^+$ and CD8$^+$ T cells are first activated independently of each other by distinct DC subsets at separate anatomical locations within the lymph node. In a second priming step, they both interact in a cognate fashion with the same XC-chemokine receptor 1 (XCR1)$^+$ lymph node-resident DC. This DC provides the platform for the delivery of CD4$^+$ T-cell help signals to the CD8$^+$ T cell.[16,17] Mouse studies show that following CD4$^+$ T-cell-mediated licensing, the ability of DCs to produce certain cytokines and co-stimulatory ligands is optimized, which serves CD8$^+$ T-cell responsiveness. DC-derived interleukin-12 (IL-12) and IL-15 play a role in inducing effector and memory CTL differentiation downstream of CD4$^+$ T-cell help.[18,19] Furthermore, co-stimulation of CD27 on CD8$^+$ T cells through CD70 on DCs is an essential downstream effect of CD4$^+$ T-cell help for survival, effector and memory differentiation of CTLs.[20–22] CD40-stimulated DCs also up-regulate CD80/86, which by stimulating CD28 on CTLs provides signals for cell cycle initiation, survival and metabolism.[23] Co-stimulatory signals through CD27 and CD28 may support CTL responses directly, and in part indirectly, via up-regulation of IL-12 and IL-2 receptors on CD8$^+$ T cells,[21,24] suggesting an interplay between cytokine and co-stimulatory signals mediated by CD4$^+$ T-cell help.

The engagement of CD4$^+$ T cells during priming can promote subsequent CD8$^+$ T-cell–DC interactions by chemokine guidance. It has been shown that after initial cognate contact with CD4$^+$ T cells, DCs start to produce CCL3/4, which attracts CCR5-expressing CD8$^+$ T cells to the site of DC–CD4$^+$ T-cell interaction.[25] Tumour-primed CD4$^+$ T cells were also shown to secrete high levels of CCL5 that recruited CCR5$^+$ DCs.[26] CD4$^+$ T cells can also facilitate the entry of naive CD8$^+$ T cells into the draining lymph nodes through the expansion of the arteriole feeding the draining lymph node.[27] Moreover, in the process termed as transphagocytosis, CD4$^+$ T cells were found to acquire, process and present antigens to naive CD8$^+$ T cells, which induced CTL memory differentiation and optimal anti-tumour responses.[28,29]

Engagement of CD4$^+$ T-cell help and the resulting CD27 co-stimulation apparently lowers the threshold for CD8$^+$ T-cell priming, as it results in broadening the T-cell receptor (TCR) repertoire of responding (tumour-reactive) CD8$^+$ T cells by the inclusion of low-affinity clones.[30–32] This is favourable for anti-tumour responses that may be of low-affinity due to negative selection of the tumour-reactive TCR repertoire in the thymus. Moreover, helped CTLs have a better cell-intrinsic ability to mediate anti-tumour responses. We have recently revealed a gene signature of helped CTLs and validated multiple molecular mechanisms by which helped CTL responses are optimized.[21] Engagement of CD4$^+$ T-cell help enhanced cytotoxic, migratory and metabolic functions of CTLs. Helped CTLs expressed higher levels of effector molecules such as tumour necrosis factor, granzyme B and IFN-γ and lower

levels of multiple co-inhibitory receptors including programmed cell death protein 1 (PD-1), lymphocyte activation gene 3 (LAG3) and B and T lymphocyte attenuator.[21,33] This resulted in more efficient killing of tumour cells. In another study, following vaccination and CD4+ T-cell depletion, the authors also identified a 'dysfunctional' state of primed CTLs. Likewise, they observed decreased expression of cytotoxic effector molecules and increased expression of multiple co-inhibitory receptors.[33] We also demonstrated that up-regulation of CXCR4, CX3CR1 and matrix metalloproteases on helped CTLs results in their enhanced migratory and invasive potential.[21] Moreover, provision of CD4+ T-cell help during priming also resulted in optimal differentiation and maintenance of tumour-specific memory CTLs.[34,35]

To efficiently mediate tumour cell killing, CTLs need to maintain their effector function. CD8+ T-cell exhaustion was originally identified during chronic viral infection in mice and characterized by progressive loss of effector functions and up-regulation of multiple inhibitory receptors.[36] T-cell exhaustion may also be related to defective memory T-cell formation, and – in the final stages – physical deletion of T cells. The observed phenotype was attributed to chronic antigen stimulation and subsequently described also in humans with cancer.[36] Importantly, the gene signature of 'helpless' CTLs resembles previously published signatures of 'exhausted' CTLs from mice suffering from chronic lymphocytic choriomeningitis virus infection.[33] Moreover, depletion of CD4+ T cells during chronic viral infection results in inability to control the virus spread.[37] The maintenance and recruitment of new virus-specific CD8+ T cells during persistent infection is impaired in the absence of MHC class II molecules[38] and adoptive transfer of virus-specific CD4+ T cells into chronically infected mice can restore proliferation and cytokine production by exhausted CD8+ T cells.[39] Interleukin-21 produced by CD4+ T cells was shown to result in extended maintenance of CTL effector functions during chronic viral infection.[40,41] Overall, these data suggest that exhausted and 'unhelped' CTLs represent two related dysfunctional T-cell phenotypes. Exhaustion is thought to be driven by chronic exposure to antigen and 'unhelped' state by insufficient priming,[36] but provision of CD4+ T-cell help may render CTLs less susceptible to subsequent exhaustion in the tissue or reinvigorate already exhausted T cells.

Overall, CD4+ T-cell help results in enhanced cell-intrinsic and -extrinsic anti-tumour activity of CTLs by optimizing their functionality during priming by multiple complementary mechanisms.

The role of Treg cells at the priming site

The Treg cells represent a distinct subset of CD4+ T cells characterized by expression of the transcription factor FOXP3 that is required for their development, maintenance and immunosuppressive function.[5,42] Treg cells have been broadly characterized as comprising two main populations: thymic or naturally occurring Treg cells (tTreg) that develop in the thymus and induced Treg (iTreg) cells that arise from mature Tconv cells. Both subsets mediate potent immunosuppressive effects and have been implicated to play a role in multiple cancer types.[43,44]

Thymic Treg cells maintain self-tolerance at steady-state by suppressing the priming capacity of DCs (Fig. 1). Treg cells continuously scan the surface of DCs and actively inhibit their maturation and interactions with naive CD4+ and CD8+ T cells.[45–47] Treg cells constitutively express co-inhibitory receptors, including CTLA-4 and LAG-3. By means of CTLA-4, Treg cells remove CD80/86 from the surface of DCs and therefore limit their co-stimulatory potential.[48] LAG-3 can bind MHC class II on DCs and thereby suppress their antigen presentation capacity.[49] Treg cells in the mouse were also shown to down-regulate CD70 from the plasma membrane of DCs in a CD27-dependent manner resulting in suppression of Tconv cell responses.[50] Other mechanisms involve down-regulation of CD40 expression and IL-12 production by DCs, resulting in attenuation of CTL effector differentiation.[51–53] Competitive consumption of IL-2 by Treg cells can reportedly limit the availability of IL-2 to early activated CD8+ T cells thereby suppressing their activation.[54]

Treg cells can also modulate chemokine production by DCs. Treg-mediated down-regulation of CCL3/4 production by DCs can inhibit attraction of CCR5+ CD8+ T cells to CD4+ T-cell–DC interaction sites[55] and thereby presumably interfere with delivery of 'help' signals. Treg cell depletion resulted in overproduction of CCL3/4, which led to stabilization of interactions between low-avidity CD8+ T cells and DCs and broadening of the tumour-specific CTL repertoire.[56] Recently, the engagement of Treg cells has been also shown to constrain the TCR repertoire of newly primed effector CD4+ T cells.[57]

Overall, tTreg cells can limit anti-tumour CTL responses by complementary mechanisms, primarily affecting the priming capacity of the DCs.

The role of Tconv cells at the effector site

Next to the role of CD4+ T cells during priming, their engagement was demonstrated to play a beneficial role also at the effector site (Fig. 2). Most tumours do not express MHC class II molecules. However, the expression of HLA-DR molecules by tumour cells has been linked to a good prognosis of cancer patients,[58,59] suggesting beneficial effects of direct recognition of tumour cells by CD4+ T cells. CD4+ T cells can acquire a cytotoxic phenotype and mediate tumour cell killing even when tumour-specific CD8+ T cells are absent.[60,61] The mechanisms involve induction of apoptosis by cytotoxic

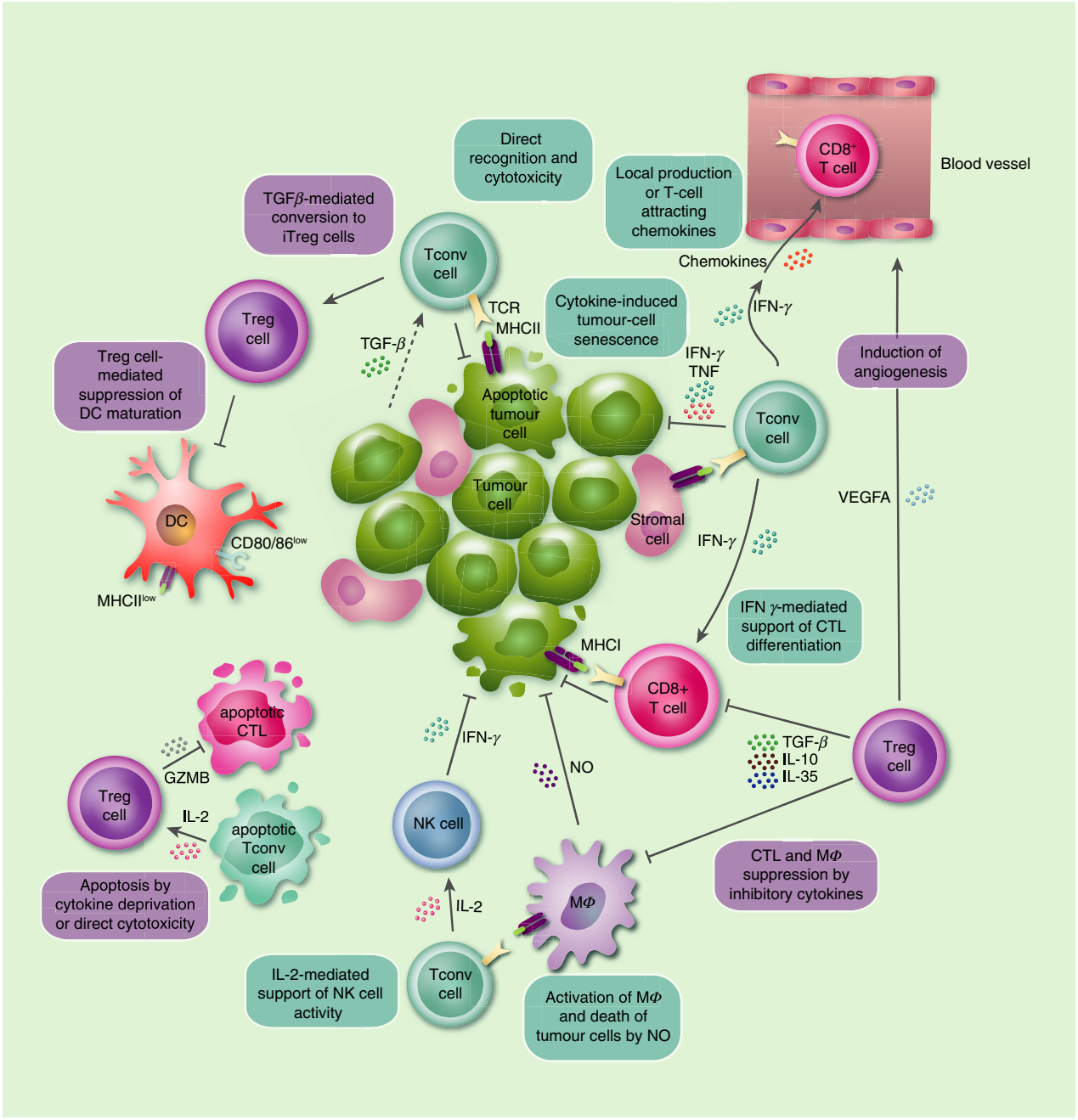

Figure 2. The roles of conventional (Tconv) and regulatory (Treg) T cells at the tumour site. Preactivated Tconv cells can directly recognize and mediate cytotoxicity against MHC class II$^+$ tumour cells. Recognition of antigen by Tconv cells on stromal cells can lead to the secretion of cytokines. Tconv cell-derived interferon-γ (IFN-γ) induces tumour-cell senescence or supports cytotoxic T lymphocyte (CTL) responses directly or indirectly by induction of cytokine secretion and attraction of new CTLs to the effector site. Activation of macrophages by Tconv cells can lead to the secretion of nitric oxide (NO) and subsequent tumour-cell killing. Tconv-derived interleukin-2 (IL-2) can support IFN-γ-mediated tumour cell killing by natural killer (NK) cells. Tumour-derived transforming growth factor-β (TGF-β) can mediate conversion of Tconv to inducible Treg (iTreg) cells. Treg cells mediate their suppressive activity by inhibiting dendritic cell (DC) maturation and induction of apoptosis of Tconv cells and CTLs by direct cytotoxicity or cytokine depravation. Treg-cell derived TGF-β, IL-10 and IL-35 inhibit CTL and macrophage responses. Local vascular endothelial growth factor A (VEGFA) production induces angiogenesis and promotes tumour progression.

granules or by stimulation via death ligands including TNF-related apoptosis-inducing ligand (TRAIL) and first apoptosis signal receptor ligand (FASL).[60] Also, in the absence of MHC class II molecules on tumour cells, their presence on the tumour stroma was shown to mediate CD4$^+$ T-cell activation and subsequent tumour

rejection.[62–64] Bystander killing of tumour cells through recognition of stroma required cooperation between CD8+ and CD4+ T cells.[65] Upon recognition of antigens at the tumour site, CD4+ T cells become activated and start producing inflammatory cytokines that can support anti-tumour immune responses by different mechanisms. CD4+ T-cell-derived IFN-γ can act directly on tumour cells, causing senescence,[66] or indirectly by enhancing CTL effector differentiation.[67,68] Production of IFN-γ can also induce local chemokine secretion and therefore enhance the entrance of CTLs to the effector site. IFN-γ-mediated secretion of CXCL9 and CXCL10 was shown to attract CXCR3-expressing CTLs from the blood into the effector tissue.[69] Conversely, aberrant expression of MHC class II by melanoma cells led to local production of tumour necrosis factor-α by CD4+ T cells and resulted in dampening of the CTL response, suggesting a negative feedback to limit unwanted CD8+ T-cell cytotoxicity.[70] Additionally, CD4+ T cells can attract and modulate the activity of multiple innate immune cell types.[71,72] For example, upon recognition of tumour-derived antigens on macrophages and eosinophils, CD4+ T cells induced their activation and production of nitric oxide and superoxide leading to tumour growth inhibition.[73] Moreover, IL-2 production by CD4+ T cells has been shown to enhance IFN-γ-mediated anti-tumour activity of natural killer cells.[74]

The role of Treg cells at the effector site

At the effector site, CD4+ Tconv cells may convert into iTreg cells due to the constitutive presence of specific T-cell or tumour-cell derived cytokines such as transforming growth factor-β.[75,76] Treg cell conversion is part of a negative feedback on chronic T-cell activation. These data suggest that effects of CD4+ T-cell help mediated at the tumour site might be limited due to their conversion into iTreg cells. In addition, tTreg cells may also reside in tumours. Expression of neuropilin 1 (Nrp1) and transcription factor Helios have been suggested to be characteristic of tTreg cells in mice and humans, respectively.[77] Due to conflicting reports regarding tTreg cell-specific markers, TCR repertoire analysis has been also used to determine the origin of intratumoral Treg cells. Several studies reported small overlap of TCR repertoire between tumour-infiltrating Tconv and Treg cells, suggesting a modest contribution of iTreg cells to the total Treg cell population.[78–80] Moreover, high frequencies of intratumoral Treg cells expressing Nrp1, have been linked to poor prognosis of patients with melanoma and head and neck cancer.[81]

Presence of Treg cells in tumours has been linked to both poor and favourable prognosis of cancer patients.[82–85] This is perhaps not surprising, as high numbers of intratumoral Treg cells can be indicative of an ongoing anti-tumour T-cell response that may ultimately be suppressed. Recently, in a model of lung cancer, Treg cells were shown to function within tumour-associated tertiary lymphoid structures to suppress anti-tumour T-cell responses.[86] Treg cells deploy multiple immunosuppressive mechanisms to keep these ongoing responses in check (Fig. 2). They can mediate apoptosis of CD8+ by direct cytotoxicity – tumour-derived factors were shown to induce grazyme B expression by Treg cells, which led to CD8 T-cell killing and diminished anti-tumour immunity.[87] Moreover, indirect induction of apoptosis by cytokine deprivation by Treg cells has been shown to limit CD4+ T-cell responses.[88] Metabolic disruption has been proposed as another mechanism of suppression mediated by Treg cells. Expression of the ectoenzymes CD39 and CD73 was shown to generate extracellular adenosine, which suppressed effector T-cell function through activation of the adenosine receptor 2A.[89] Additionally, adenosine has been suggested to play a role in further up-regulation of Treg cell suppressor functions.[90] Inhibitory cytokines have been the focus of substantial attention as mediators of Treg cell-mediated suppression at the effector site. Interleukin-10 and transforming growth factor-β were shown to inhibit tumour-specific T-cell infiltration and effector function and to promote an anti-inflammatory phenotype in macrophages.[91–93]

Intratumoral Treg cells were also shown to play a role in the loss of effector function by CD8+ T cells. Recently, Treg-cell-derived IL-35 was shown to promote effector T-cell exhaustion within the tumour microenvironment.[94] Treg cells were also shown to induce down-regulation of effector molecules and up-regulation of inhibitory receptors on CD8+ T cells. This process was dependent on reduction of co-stimulatory potential of intratumoral DCs. Depletion of Treg cells led to rescue and expansion of 'exhausted' tumour-specific CTLs.[95] In a model of chronic virus infection, a similar effect was dependent on the provision of CD4+ T-cell help and co-stimulatory signals to the exhausted CTLs, highlighting the complementary roles of Tconv and Treg cells in maintaining functional CTL responses.[96] Treg cells have also been suggested to contribute to tumour progression by inducing angiogenesis. Hypoxic tumours were shown to attract Treg cells in a CCL28-dependent manner, which resulted in increased production of vascular endothelial growth factor A.[97]

On the other hand, Treg cells were shown to promote generation of memory CD8+ T cells.[98] Expression of CTLA-4 and production of IL-10 by Treg cells have been shown to play a role during the contraction and resolution phase to promote memory CD8+ T-cell formation through the suppression of pro-inflammatory cytokine production by DCs.[99,100]

Implications for cancer immunotherapy

The goal of cancer immunotherapy is to elicit an effective CTL response. This can be achieved by reactivating pre-

existing tumour-specific CTLs and/or by priming of naive tumour-specific CD8$^+$ T cells. In an ideal scenario, cancer immunotherapy initiates and supports a 'cancer immunity cycle' wherein the cancer acts as its own vaccine.[101] Cancer cell killing releases tumour antigens that are presented to naive T cells in secondary lymphoid organs. As DC-activating 'danger' signals are generally lacking in this scenario, appropriate therapeutic intervention may enhance priming of new CTLs and in turn increase the range of antigens that is recognized. The quality of tumour-specific CTL responses is modulated by both Tconv and Treg cells, as outlined above. It is therefore important to know how various cancer immunotherapy approaches affect both cell subsets. Current strategies involve therapeutic vaccination, treatment with immunomodulatory antibodies and adoptive cell transfer.

Early studies in mice showed that vaccination with small peptides that directly bind to MHC class I molecules induced CD8$^+$ T-cell tolerance, which could be overcome with agonistic anti-CD40 antibody and resulted in tumour-specific CTL responses.[102] Follow-up studies demonstrated that vaccination with long peptides encompassing both MHC class I and class II epitopes induced optimal CTL-based anti-tumour immunity.[103] Subsequently, inclusion of MHC class II epitopes in therapeutic vaccines has been shown to improve CTL responses and survival of patients with melanoma or vulvar neoplasia.[104–106] Vaccination with mutant MHC class II epitopes was shown to drive the therapeutic response to established mouse tumours by inducing CD4$^+$ T-cell responses. Observed effective anti-tumour CTL activity was linked in part to the decrease in intratumoral Treg cells following the vaccination.[107] Recent studies in melanoma patients demonstrated that vaccination with a synthetic long-peptide or neo-antigen bearing RNA-transfected DCs resulted in potent CD4$^+$ T-cell responses and durable tumour control.[12,13] Depletion of Treg cells might further improve the responses to vaccination. Application of recombinant IL-2–diphtheria toxin conjugate, resulting in Treg cell depletion, has been shown to enhance CTL responses following vaccination with RNA-transfected DCs.[108,109] Modulation of Treg cell activity can be also achieved by rational vaccine design. Vaccination with an NY-ESO-1 peptide inducing newly primed low-avidity Tconv cells did not induce an antigen-specific Treg cell response and therefore undermined the suppressive activity of Treg cells on high-avidity NY-ESO1-specific T-cell precursors.[110]

The most successful and widely applied form of cancer immunotherapy makes use of blocking antibodies to co-inhibitory receptors CTLA-4 and/or PD-1. Clinical benefit observed after the treatment with these antibodies has been attributed to the effect on anti-tumour CTL responses. However, the final outcome might be partly due to indirect effects through opposing the activity of Treg cells and promoting the activity of Tconv cells as suggested by expression of CTLA-4 and PD-1 by both cell subsets.[111]

Treatment with anti-CTLA-4 monoclonal antibody was shown to induce priming of a new T-cell response against the tumour and thereby broadening of the repertoire of melanoma-reactive CD8$^+$ T cells.[112] These data suggest that CTLA-4 blockade overrules peripheral tolerance, in line with the fact that in the mouse, both effector and regulatory T-cell compartments contribute to the anti-tumour activity of anti-CTLA-4 antibodies.[113] CTLA-4 blockade and Treg cell depletion also synergized in support of an anti-tumour CTL response, which could be explained by the fact that Treg cells use multiple CTLA-4-independent immunosuppressive mechanisms.[114] Importantly, the inhibitory and depleting properties of anti-CTLA-4 antibodies have been shown to depend on their isotypes.[115]

Blocking PD-1 or its ligand PD-L1 seems to primarily overrule the suppression exerted on pre-existing tumour-specific CTLs.[116] It enables co-stimulation via CD28 and likely engages the activity of CD4$^+$ and CD8$^+$ effector T cells, as was demonstrated in mouse models.[117,118] More recently, the response to PD-1 treatment also proved to depend on IFN-γ-mediated suppression of Treg cell activity.[81] Similarly to CTLA-4 blockade, treatment with anti-PD-1 antibodies synergized with Treg cell depletion to mediate tumour regression in mouse melanoma model.[119]

Combination of CTLA-4 and PD-1 showed synergistic effects in melanoma patients, suggesting complementary roles of both receptors.[120] Both strategies were shown to induce proliferation of specific subsets of CD8$^+$ T cells with 'exhausted-like' phenotype. CTLA-4 blockade additionally induced expansion of a subset of effector CD4$^+$ T cells, possibly enabling the effects of CD4$^+$ T-cell help at the tumour site.[121]

Anti-tumour effects of adoptive cell transfer have been shown to depend on both Tconv and Treg cell responses.[122] Durable clinical remissions have been observed in patients with metastatic melanoma treated with autologous CD4$^+$ T cells against NY-ESO-1.[123] Additionally, vaccination with MHC class II epitopes induced better tumour control and allowed for the reduction in the number of adoptively transferred CD8$^+$ T cells needed to protect against mouse tumour challenge.[124]

Conclusions

Treg and Tconv cells play important and complementary roles in CD8$^+$ T-cell function. This is relevant for both autoimmunity and anti-tumour immunity. The occurrence of autoimmunity depends on lack of immunosuppression by Treg cells, as well as on ongoing interactions between Tconv cells and antigen-presenting cells maintaining CTL effector function.[125] Anti-tumour CTL

responses are often improved upon the depletion of Treg cells and engagement of Tconv cells. Treg cells mediate their suppressive activity by directly opposing the beneficial effects provided by CD4+ T-cell help. This is supported by the fact that depletion of Treg cells can promote tumour-specific CD4+ T-cell responses and that CD4+ T cells can counteract Treg cell-mediated suppression via CD40L–CD40 interaction with DCs.[126,127] We therefore propose that engagement of Treg cells prevents optimal delivery of CD4+ T-cell help both during priming and at the effector site.

Moreover, engagement of Treg cells and lack of CD4+ T-cell help have been shown to result in up-regulation of inhibitory receptors on antigen-specific CTLs.[21,94,98] Tumour-specific CD8+ T cells present in the blood of melanoma patients express PD-1.[128] Circulating PD-1+ CD8+ T cells in cancer patients may therefore represent the 'helpless' phenotype, as tumours may not offer the required innate DC-activating signals and promote Treg cell responses that prevent optimal delivery of CD4+ T-cell help signals as discussed above. Treg and Tconv cells use different signalling pathways downstream from TCR stimulation and rely on different metabolic pathways for optimal responses.[129,130] Such insights into cell-intrinsic differences of Tconv and Treg cells may help to specifically modulate each subset to promote anti-tumour immunity. Therefore, cancer immunotherapy strategies that differentially affect each subset may prove to be most efficient.

Type I IFN has been shown to limit Treg responses and induce downstream effects of CD4+ T-cell help.[8,131] Therefore, promising approaches inducing type I IFN production include radiotherapy or stimulator of interferon genes (STING) agonists in combination with vaccination or antibodies against PD-(L)1. Another promising candidate is CD27 agonist antibody. In our mouse model of therapeutic vaccination, CD27 agonism and PD-1 blockade together recapitulated the effects of CD4+ T-cell help.[35] Anti-tumour efficacy of CD27 agonist antibody has been shown to depend on effector T-cell stimulation as well as Treg cell depletion.[132] First-in-class anti-CD27 antibody has proven safe in a recent phase 1 clinical trial.[133]

Overall, all current cancer immunotherapy strategies rely in part on immunomodulatory effects mediated by Tconv and Treg cells. Further mechanistic insight into these cell subsets will allow for the manipulation of their activities and subsequently improve the clinical responses of cancer patients.

Disclosures

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