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Challenges and future perspectives of T cell immunotherapy in cancer

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Abstract

Since the formulation of the tumour immunosurveillance theory, considerable focus has been on enhancing the effectiveness of host antitumour immunity, particularly with respect to T cells. A cancer evades or alters the host immune response by various ways to ensure its development and survival. These include modifications of the immune cell metabolism and T cell signaling. An inhibitory cytokine milieu in the tumour microenvironment also leads to immune suppression and tumour progression within a host. This review traces the development in the field and attempts to summarize the hurdles that the approach of adoptive T cell immunotherapy against cancer faces, and discusses the conditions that must be improved to allow effective eradication of cancer.

Keywords

Cancer immunotherapy; Tumour escape; T lymphocyte; Immunosuppression; Immunoediting; Immune activation; Checkpoint inhibition

1. Introduction

The immune system has evolved in multicellular organisms as a guard against foreign infections that threaten host survival. Most clinically manifested tumours originate from transformed normal cells. Moreover, many of the identified tumour antigens correspond to self MHC-associated peptide fragments of self-proteins. This has presented problems for

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optimal stimulation of T lymphocytes against tumour cells in both preventive and therapeutic settings. The efforts of developing an effective T cell therapy of cancer have thus been extremely difficult and slow.

Encouragingly, in some models, the T cell mediated immune response can efficiently control tumour. In the last two decades, a large number of antigens have been identified on tumours that can be recognized by T cells [1–4], suggesting a potential role of T lymphocytes in anti-tumour immune response. Functional cytolytic T cells (CTL) were shown to develop against tumour cells expressing self-tumour antigens in some melanoma patients [5,6], pancreatic cancer patients [7], Epstein-bar virus-associated malignancies [8] and murine tumour models [9–13]. Interestingly, activated CTLs with sufficient avidity to kill breast cancer cells were found to be present in breast cancer patients [14]. Intriguingly, in a melanoma model, the tumour-bearing state was shown to reverse the tolerance of T cells [15,16]. An adoptive cell therapy regimen comprising autologous tumour-infiltrating cells or TCR gene transduced cells capable of recognising gp-100 or MART-1 antigens could abrogate melanoma metastasis to brain with long term remission [17]. Also, a “safety switch” was suggested for adoptive cell therapy in leukemia patients, whereby it became possible to quickly eliminate the transplanted T cells in case an adverse reaction like GVHD developed [18]. These observations rekindled hopes for the potential of tumour antigen vaccination and adoptive cell transfer protocols to enhance anti-tumour T cell responses. Autologous dendritic cells pulsed with tumour peptides or whole tumour lysates were shown to stimulate T cell responses in several tumours, including gliomas and melanomas [19–22].

Paradoxically, in some models, the T cell receptor (TCR) transgenic mice replete with high frequency of tumour antigen-reactive monoclonal T cells or the generation of a large number of T cells by vaccination or transfer of monoclonal T cells did not result in significant tumour regression [23,24] and the T cell response, if any, was somehow compromised. T cells seemed to fail particularly in the context of a large tumour burden [25]. Is this failure of T cells due to (a) an outnumbering of T cells by a progressive burden of tumour mass, or (b) to the defects *per se* in T cell structural and functional attributes attained during the course of T cell-tumour interaction, or (c) to deficiencies in the maintenance of sustained tumour-specific T cell activation, or (d) to a lack of concerted help from other immune cells or (e) to a suppression caused by other immune-suppressive factors or cells in the tumour microenvironment? These outcomes may be the result of direct tumour-T cell cross-talk or due to indirect effects of inflammation or cellular stress associated with tumourigenesis. This review attempts to discuss the various factors that compromise the anti-tumour response of T cells as summarized in Table 1. Taking into account the recent attempts like genetic engineering of T cells, and hitherto latent promising effects of T cells as shown in mouse models of T cell immunotherapy, the authors build up on the conditions that may be improved in order to favour the success of adoptive cell immunotherapy of cancer.

2. Bystander effects of tumourigenesis

2.1. Alterations in the T cell signal-transducing TCR-CD3 complex

Earlier studies showed that the tumour growth may cause T cell dysfunction by inducing abnormalities in the TCR-CD3 complex resulting in an unproductive signal transduction

following TCR: MHC-peptide ligation. Alternatively, tumour growth may decrease the dissociation of TCR: MHC-peptide complex compromising the efficiency of antigen recognition by CTL and lysis of tumour cells [26,27]. Altered T cell signal transduction has been reported in a variety of tumours, including renal, colorectal, ovarian, liver, gastric, oral, prostate, pancreatic and cervical carcinomas, glioblastomas and melanomas (Table 2). The decreased CD3 ζ chain in T cells is a correlate of poor prognosis or survival of patients with cancer [28,29]. Reduced levels of protein and mRNA expression of the Src-family protein tyrosine kinases (PTKs) p^{56lck} and p^{59fyn} and the ζ chain in the TCR-CD3 complex have been reported in the murine tumour models with the concomitant increase in the expression of Fc ϵ RI γ chain, and/ or blunted Ca²⁺ flux [30,31]. Lower CD3 ζ chain levels caused a reduced surface TCR expression preventing optimal T cell activation and proliferation in response to the cognate peptide-MHC [30,32]. Similarly, in humans bearing renal, hepatic colorectal, head and neck squamous cell carcinomas, also melanoma and B-cell lymphoma, tumour-infiltrating T lymphocytes (TILs) and peripheral blood lymphocytes (PBLs) showed decreased protein tyrosine phosphorylation and diminished protein levels of the CD3 ζ chain, p^{56lck} and ZAP-70 tumour when compared with healthy controls [31,33–39].

However, the reports documenting tumour-induced changes in the TCR-CD3 signal transducing complex came under criticism when Franco J.L. et al [40] showed that the majority of the reduction in CD3 ζ -chain and part of the reduction in p^{56lck} was due to the degradation of these proteins by the contaminating granulocyte proteases in the enriched T cell populations during protein extraction *ex vivo* [40]. Nevertheless, the tumour-induced abnormality in the TCR-CD3 signal transduction still held true as the downstream signalling molecules NF- κ B p⁶⁵ and c-Rel were detected at reduced levels in tumour-bearing mice and patients showing renal carcinoma and other pathological conditions [40–43]. The blockade of T cell signal transduction c-Myc and pRb pathways as well as inhibition of nuclear translocation of NFATc and NF- κ B were observed in the presence of acute myeloid leukaemia [44,45]. Therefore, tumour growth may affect T cell signal transduction but whether it occurs due to changes in the signals regulating the nuclear transcription factors or to TCR structural changes still remain to be determined.

An alternative view is that the tumour-induced perturbations in the T cell signal transduction may only be a transient phenomenon. Indeed, in the situations of low tumour burden, the decreased levels of NF κ B, CD3 ζ , and p^{56lck} proteins in splenic T cells were reversed following flavone 8-acetic acid and recombinant IL-2 therapy of renal carcinoma in mice [41]. Similarly, peripheral blood lymphocytes of prostate cancer patients were shown to regain normal CD3 ζ levels after 48 hours culture *in vitro* in serum free medium [46]. CD3 ζ chain down-modulation may thus only be a physiological response to attenuate an exacerbated immune response to the continuous presence of tumour antigens and the associated chronic inflammation. This situation may be analogous to a chronic infection where a loss of CD3 ζ chain by an IFN- γ -dependent lysosomal degradation was observed after repeated exposure to various non-specific antigens that generate an inflammatory response [47]. The tumour growth-associated chronic inflammation may thus be the major culprit for inducing alterations in the signal transducing TCR-CD3 complex. Indeed, in an inducible melanoma model, tumour-associated chronic inflammation blunted the protective

anti-tumour T cell immunity [48], which could be corrected by engineered expression of transcription factor STAT5 in antigen-experienced CD8 T cells [49] Additional studies are thus warranted to investigate T cell signal transduction in a wide spectrum of tumours in relation to attenuation of T cell stimulation

2.2. Induction of T cell tolerance

A large majority of human tumours constitutively express indoleamine 2, 3-dioxygenase (IDO) [50] which has been implicated in the catabolism of the essential amino acid tryptophan in macrophages and dendritic cells and has been shown to regulate the adaptive T cell response [51–57]. IFN γ induces the expression of IDO and inducible Nitric Oxide Synthase (iNOS) on the surface of macrophages which in turn catabolize tryptophan and arginine respectively [58,59]. Tryptophan deprivation sensitizes activated T cells to apoptosis [60] thereby eliminating anti-tumour T cells [61]. Silencing of tumour-associated DCs that induce tolerance in T cells through overexpression of FOXO3 was found to inhibit the expression of IDO, arginase and TGF- β [62]. The role of IDO in T cell tolerance to tumours has been extensively reviewed [63,64]. The tolerogenic effect of IDO was observed when originally immunogenic mouse failed to reject tumour cells after induction of IDO expression. Moreover, IDO being an IFN γ -inducible enzyme, IDO negative tumour cells may also start expressing IDO when exposed to an inflammatory context, decreasing the efficacy of immune response. Recently, pharmacological inhibition of the IDO pathway by using stereoisomers of l-methyl-tryptophan has been on the rise as a therapeutic modality in combination with immunotherapy against cancer [65]. However the role of IDO activity is not always correlated to anti-tumour immune suppression, in patients with gynecological cancers as tryptophan degradation and IDO activity have been shown to augment immune activation rather than suppress it [66]. Future studies will be required to understand how IDO may be regulated to promote anti-tumour T cell response.

T cell suppression can also be modulated through immature CD11b⁺Ly6G⁻ monocytes from the blood of naïve mice. When these immature monocytes synapse with T cells, they suppress their activity by cell contact in a mechanism partially NOS-dependent but completely IFN γ -independent [67] Macrophages can also suppress T cells activation by producing reactive oxygen species (ROS) in an antigen-dependent manner, diminishing T cell proliferation and IL-2 production. [68]. In solid tumours, induction of hypoxia by macrophages through hypoxia inducible factor-1 α (HIF-1 α) leads to T cell suppression. Ablation of HIF-1 α slowed tumour progression and increased anti-tumour T cell response [68,69] , showing an interconnection between adaptive and innate immune responses during tumour-induced T cell suppression.

2.3. Shedding of gangliosides by tumour

Tumour progression, either *in situ* or metastatic, is dependent on subtle interactions between tumour cell and host cell membrane domains, termed 'glycosynapses', involved in glycosylation-dependent cell adhesion and signalling. Tumour-associated gangliosides, organized in glycosynapses, function as adhesion receptors with consequent activation of signal transducers leading to enhanced tumour cell motility and invasiveness [70–73]. Ganglioside structures may cause deletion or suppression of immune cells and promote

tumour angiogenesis and metastasis. They are directly connected with transducer molecules in the lipid raft microdomains to initiate adhesion coupled signalling through interaction with tyrosine kinases associated with growth factor receptors or other protein kinases [74,75]. Tumour-derived gangliosides have also been shown to impair dendritic cell differentiation and maturation [76,77]. Gangliosides have been shown to mediate degradation of RelA(p65) and p50 protein levels in tumour-infiltrating T cells in renal cell carcinomas leading to defective NF κ B activation and a heightened T cell sensitivity to apoptosis [78]. Gangliosides, GM1 and GD3, shed off by a T cell lymphoma have been shown to inhibit bone marrow cell proliferation and differentiation, induce apoptosis in bone marrow cells, and inhibit nitric oxide production from macrophages [79–81]. In a malignant melanoma model GD-3-mediated tumour cell growth and invasion was shown to be affected by the molecules p130Cas and paxillin using small interfering RNA (SiRNA) approach [82].

Given the fact that gangliosides may act as onconeural antigens and modulate biological behavior of immune cells *in vivo*, further studies as to how gangliosides can be moulded into immunopotentiatory molecules are warranted. Ganglioside based-bioengineering approach for cancer therapy of human tumours has been attempted [83] and evaluated in melanoma [84,85] and glioma [86]. In patients with melanoma, a phase I/II clinical trial was conducted to evaluate efficacy of a vaccine using NGcGM3/very-small-size proteoliposomes (VSSP). All patients developed IgG and IgM antibodies against NeuGcGM3, and overall had a reasonable anti-tumour response with survival rate higher than previously reported in the literature [85,87]. The vaccine showed to be safe, immunogenic and efficacious against metastatic melanoma.

2.4. Production of immunosuppressive cytokines and factors by tumour

Since the first demonstration that transforming growth factor β -1 (TGF β -1) secreted by immunogenic tumours may promote tumour escape from T cell cytolytic immune response [88] by its pleiotropic effects on T cell proliferation, differentiation, cytokine production and antigen presentation, an impressive variety of immunosuppressive and tumour promoting cytokines or factors, ranging from acute phase reactants with non-specific inhibitory properties, to adhesion molecules blocking cell interactions or apoptosis have been reported. It has been demonstrated that T cell-specific blockade of TGF- β signalling allows the generation of an immune response capable of eradicating tumours in mice challenged with B6 or EL4 tumour cells [89,90].

Increase in the soluble forms of T cell interaction molecules such as intracellular adhesion molecule (ICAM/CD54), lymphocyte function-associated antigen-3 (LFA-3/CD58), and others correlate with tumour progression [91–93]. Soluble Fas ligand, Annexin II, MUC-1 and MUC-2 molecules from tumour may delete or suppress T cells [94–97]. Shedding of FasL-containing membrane vesicles from ovarian tumours has been observed and shown to suppress TCR/CD3 ζ chain expression and cause T cell apoptosis in patients [98]. Secreted FasL was thus proposed as a mediator of the tumour “counterattack” on T cells [99]. In advanced gastric carcinoma and human melanoma, FasL and APO2L/TRAIL have been ascribed to contribute to tumour counterattack [100,101]. In colon cancer, down-regulation of FasL expression by colon tumour cells results in an improved antitumour immune

challenge *in vivo*, favouring the "Fas counterattack" model as a mechanism of tumour immune evasion [102]. Fas/FasL system presents an important mechanism of tumour-T cell interaction that needs to be fine-tuned to avail anti-cancer effects. However, in some animal tumour models FasL expression may result not in escape, but in rather more rapid rejection [103]. It has been observed that growing tumours under the conditions of high levels of IFN γ overexpress Fas and become susceptible to FasL expressed on T cells [104].

Tumours may also escape from immune detection by producing "unopposed" GM-CSF and soluble vascular endothelial growth factor (VEGF), thereby disrupting the balance of cytokines needed for the maturation of fully functional antigen presenting cells [105,106] and Notch1 signaling crucial for effector lymphocyte differentiation [107]. IL-10 is another major immune dysregulating cytokine secreted by various solid tumours, as well as haematological malignancies [108,109], carcinomas [110] and chronic myeloid leukemia [111]. IL-10 has been shown to hinder a number of immune functions, including T lymphocyte proliferation, pro-inflammatory T_H1-type cytokine production, antigen presentation, and lymphokine-activated killer cell cytotoxicity. There is also evidence that IL-10 can enhance tumour rejection [112], elicit T cell memory due to the combined action of NK cells, CD8⁺ T cells and neutrophils [113], and, in conjunction with CD80-CD28 costimulation, can prime tumour-reactive T cells [114]. This may be due to the ability of IL-10 to prevent T cell apoptosis [115,116]. Moreover, DCs exposed to IL-10 induce anergy in tumour peptide-specific T cells [117], and prime IL-4-secreting T cells, perhaps by default due to the down-regulation of IL-12 production and reduced expression of costimulatory molecules [118]. In addition, IL-10-exposed DCs are more susceptible to lysis by autologous NK cells [119]. Transgenic mice expressing IL-10, however, did not limit the growth of immunogenic tumours [120]. Signalling through IL-10R α on DCs seems to have a strong immunosuppressant effect on CD8⁺ T cells from tumour bearing mice. Silencing IL-10R α on DCs by using siRNA was able to increase anti-tumour immune response on CD8⁺ T cells and when in combination with siTGF- β R, higher frequencies of antigen specific CD8⁺ CTLs and anti-tumour response were found in a cervical cancer model [121]. On the other hand, IL-10 has been shown to exert anti-angiogenic and anti-metastatic effects in certain murine models [122]. A vaccination using vaccinia virus armed with murine IL-10 showed promising results as increased survival and anti-tumour immune response, besides better tumour reduction in a murine pancreatic model. Although the mechanisms behind IL-10 effect on improved anti-tumour response were not completely understood, this cytokine showed anti-tumour properties [123]. Due to the counteracting effects that IL-10 can exert, it remains difficult to predict the net effect of this cytokine on host antitumour mechanisms and needs to be further examined carefully.

3. Modulation of immune cells in tumour microenvironment

3.1. Tumour-induced production of reactive oxygen and nitrogen metabolites, IL-1 and arginase by tumour-infiltrating immune cells

Tumour microenvironment has been shown to "educate" myeloid cells to change their redox potential so that they produce compounds ranging from mutagenic oxygen and nitrogen radicals to angiogenic factors that can promote tumour growth and metastasis.

Inflammation-induced recruitment of non-T cell populations such as macrophages [124,125], granulocytes or natural killer cells [126], CD11b⁺Gr-1⁺ myeloid suppressor cells [127,128] or dendritic cells [129] have been shown to occur in the spleen or tumour-draining lymph nodes [130,131]. These non-T cells secrete reactive oxygen metabolites that induce CD3 ζ chain down-regulation [124,131]. In a T cell lymphoma model, tumour-associated macrophages were shown to exhibit an increased production of reactive nitrogen intermediates [132] and an enhanced IL-1 production [133]. Co-culture of macrophages from the spleens of tumour-bearing animals with normal T cells caused disappearance of the T cell CD3 ζ chain through oxidative stress [131,134]. Alternatively, the consumption of L-arginine, a non-essential amino acid, by tumour-associated macrophages may cause a reduction in extracellular levels of L-arginine, decrease the expression of CD3 ζ chain and diminish T cell function [133,135,136].

Thus, the hypoxic milieu and the associated oxidative stress that develops in the vicinity of tumour act as a two-edged sword by modulating the immune cells in such a way that they produce hypoxia-inducible factors, causing a suppression of anti-tumour immune response and promoting tumour growth. HIFs have also been shown to regulate the survival of antigen-driven T cells [137]. This brings out the importance of controlling tumour microenvironment for any successful tumour therapy.

3.2. Tumour-induced production of immunosuppressive cytokines by immune cells

Tumours can exert nonspecific suppressive effect as a result of their hypoxic metabolism; for example, by secreting adenosine they can inhibit IL-12 and stimulate IL-10 production by monocytes [138,139]. There is also evidence that other cytokines, such as circulating IL-6, are associated with decreased survival and increased extent of metastatic breast cancer [140]. IL-6 production could contribute to peripheral T lymphocyte dysfunction, enabling tumour cells to escape immune surveillance by preventing specifically the antitumour T_H1 immune responses [141]. Tumours can also induce T_H2 cells to produce IL-10 that can contribute to immunosuppressive effects and the MHC class I and II down-regulation on tumour cells. Another cytokine IL-13, possibly produced by NKT cells, signals through the IL-4 receptor-STAT6 pathway, and down-regulates T cell anti-tumour responses, facilitating tumor recurrence [142]. Inhibiting these immunosuppressive cytokines in combination with other immunotherapy regimen may prove to be useful in cancer immunotherapy.

3.3. Tumour-induced immune imbalance by increased numbers of immature or immunosuppressive immune cells

Tumour growth negatively affects myelopoiesis by inhibiting the process of differentiation/maturation of antigen-presenting cells from their myeloid precursors and by stimulating an accumulation of immature myeloid cells observed both in cancer patients and tumour-bearing mice [143]. These immature myeloid cells can contribute greatly to tumour progression by migrating into the tumour site and causing an immune imbalance. They differentiate into highly immunosuppressive immune cells and mediate the development of tumour-induced T regulatory (T_{Reg}) cells and T cell anergy [130,143,144]. The immature immune cells can comprise a small percentage of hemopoietic progenitor cells, and a majority of immature macrophages and DC, or immature myeloid cells at earlier stages of

differentiation. The immature myeloid cells are able to directly inhibit Ag-specific T cell responses in lymphoid organs. In mice, chronic GM-CSF production by tumours increased an inhibitory population of adherent CD11b+Gr-1⁺ cells of "immature" myeloid phenotype that suppressed Ag-specific CD8⁺ T cell responses upon direct cell-to-cell contact [145].

In bacterial and viral infections, the immature dendritic cells residing in peripheral tissues are efficiently activated and matured by pathogen signals for performing the immune response. In contrast, in the presence of tumour-associated self-antigens expressed on tumours, the DCs do not mature enough to activate the naive T cells, but rather cause their anergy/deletion, and lead to the generation of T_{Reg} cells setting in immune tolerance. Tumour cells are thus able to exploit the functional roles of immature DCs for tumour progression and evasion from immune attack. The strategies to target immature DCs should potentially provoke tumour immunity [105].

In addition to immature myeloid suppressor cells, active suppression of incipient "self" responses elicited by tumour antigens can also occur by CD25⁺FoxP-3+CD4⁺ T_{Reg} cells, $\gamma\delta$ T cells, $\alpha\beta^{\text{int}}$ T cells [146], IL-13 secreting NKT cells [142], CD8⁺ T suppressor cells [147,148]. Balancing the immune suppression posed by these suppressor cells with the activated effector T cells is required to be able to mount an optimal anti-tumour response. We have discussed T_{Reg} cells in more detail in later section of this review.

4. Modification of T cell recognition and signal strength

4.1. Direct effect of tumour on T cell recognition

In mouse models as well as patients, tumours have been found to directly compromise T cell recognition by altering their surface expression of HLA and MHC molecules [149,150]. In some instances, tumour cells completely lack all HLA-class I molecules. In other cases, there is a loss of a single allele, of one full haplotype or of the products of a given HLA locus. Some tumours show loss of β 2-microglobulin (β 2m), an essential and invariant subunit of class I MHC complexes, as a mechanism of escape from T cell recognition. However, in animal models and human melanoma cells, it has also been demonstrated that the loss of β 2m results in exquisite sensitivity to NK cell-mediated killing [151] and leads to tumour elimination, not escape [152,153]. Loss of the MHC class I heavy chain or transporters associated with antigen processing or low-molecular-weight proteins complex components have also been observed in some tumours and human melanoma [154]. No increased tumour incidence was however observed in mice lacking TAP-1, a subunit of the transporter for antigen presentation, or LMP-2, a regulated subunit of the 20S proteasome, nor was the relative incidence of lymphomas versus sarcomas altered by variation in TAP-1 or LMP-2 [155]. Nonetheless, a reduced expression of the TAP-1/2 proteins may be responsible for the decreased MHC class I presentation. Inhibition of IRF-1 and TAP-1 was also observed after transfection of Human Papillomavirus 16 (HPV16) E7 protein on mouse keratinocytes, leading to lower MHC class I expression induced by IFN- γ . This mechanism is used by HPV to escape immune surveillance and facilitate virus survival on cervical cells, which increases the risks of cervical carcinoma in women infected by this virus.[156]. In addition, it has been demonstrated that TNF- β , in concert with IFN- γ , increased or restored

HLA molecules in acute myelogenous leukemia (AML) increasing the interactions between AML blasts and T cells [157].

4.2. Inhibition of T cell function by negative immunoregulatory receptors on tumours

Tumour cells may lack the expression of costimulatory molecules including B7-1/CD80, B7-2/CD86 and CD40 ligand. Transfection of mouse tumours with costimulatory molecules has been used to trigger their immune-mediated rejection. However, rejection is not observed when B7 molecules are inserted into less immunogenic tumours [158]. A greater understanding of the interactions of costimulatory molecules with negative regulatory molecules may enable more directed therapeutic interventions [159–161]. The interaction of costimulatory molecules on tumour cells with cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) can inhibit T cell function by blocking TCR-CD3-mediated signal [162].

B7 homolog 1 (B7-H1/PD-L1), a B7 family member and ligand for programmed death-1 (PD-1), an immunoinhibitory member of the CD28 family is expressed on a majority of human cancers including melanomas and carcinomas of lung, ovary and colon. It is also expressed on the activated lymphocytes, antigen presenting cells and myeloid cells [163–165]. PD-L1 can interact with receptors on CTLs and promote apoptosis of effector CTLs via induction of FasL and IL-10. It has also been shown that the engagement of PD-1 receptor leads to recruitment of SHP-2, which can dephosphorylate TCR proximal kinases, leading to a reduced TCR signal [164]. Other studies have also suggested that PD-1 engagement results in inhibition of antigen cross-presentation, T cell proliferation, reduced cytokine production and cell-cycle arrest [164,166]. Antibodies against PD-L1 enhanced killing of tumour cells by protecting CTLs from PD-L1-induced death. Notably, IFN- γ , a key cytokine produced by CTLs amongst other cells, was found to induce expression of PD-L1 on many tumour cell lines. As a repercussion of this, the effector CTLs may thus hasten their own demise by production of IFN- γ that in turn upregulate PD-L1 expression on tumours [163].

Various other molecules that negatively regulate the immune response, such as the CD200, a type-I membrane glycoprotein, are found to be upregulated on various tumours. Targeted anti-CD200 immunotherapy is being tested in clinical trials [167]. The blockade of CD200 glycoprotein has been shown to significantly enhance T cell proliferation in vitro, stimulate T cell responses against fibromodulin, a chronic lymphocytic leukemia (CLL) associated antigen, and reduce the number of CD4⁺/CD25^{hi}/FOXP3⁺ T cells (T_{Reg}) cells [168]. Additionally, the use of siRNAs and mAb against CD200 could suppress CD200 function with a concomitant increase in the production of IFN- γ and TNF- α by effector PBMCs [169]. Similar T cell inhibitory effects have been documented for CD244 (2B4), a signaling lymphocyte activation molecule (SLAM) family receptor, whose expression is found on natural killer (NK) cells, $\gamma\delta$ T cells, activated CD8⁺ T cells, monocytes and basophils [170,171]. The presence of CD244 on T cells from lung tumor bearing mice correlated with increased apoptosis and exhaustion, contributing to tumor resistance [171]. In a mouse model of melanoma, two vaccines expressing CD4⁺ and CD8⁺ T cells epitopes for melanoma developed different anti-tumor responses. The vaccine expressing HSV-1 glycoprotein D (gD) was able to delay tumor growth due to its ability in lowering expression

of inhibitory molecules as 2B4 on tumor specific CD8⁺ T cells, suggesting the presence of gD can overcome tumor-driven exhaustion of tumor specific T cells [172].

Other prominent T cell regulatory proteins include T cell immunoglobulin domain and mucin domain 3 (TIM3), B- and T-lymphocyte attenuator (BTLA), CD160, lymphocyte-activation gene 3 (LAG3) and the unique killer cell lectin-like receptor subfamily G member 1 (KLRG1). Along with negative regulation of T helper 1 cells, Zhou and colleagues have reported an “exhaustion” phenotype of CD8⁺ T cells which co-express Tim-3 and PD-1. Simultaneous blockade of PD-1/PDL1 and TIM-3/galectin-9 pathways could prevent T cell exhaustion as these cells were more resistant to AML when compared with cells in which only one pathway was blocked [173,174]. Contrary to this, however, Galectin-9 expands Tim3⁺ dendritic cells which interact with CD8 T cells to enhance their antitumour effector functions on methylcholanthrene-induced sarcoma (Meth-A) tumor bearing mice [175]. It has been shown that Tim-3 stimulates TNF production in naïve DCs to induce NF-κB and enhance a pro-inflammatory response [176]. BTLA and the relatively newly discovered CD160 [177] share a common ligand, the HVEM (herpesvirus entry mediator), which also binds LIGHT, another costimulatory molecule that positively regulates T cell response. However, HVEM can function bidirectionally, as a positive or negative signal transducer, depending upon the receptor it binds [178,179]. BTLA expression is downregulated by virus specific CD8 T cells but not by tumour specific cells. Therefore, suitable immunotherapeutic approaches like vaccination with CpG have been suggested [180]. Blockade of BTLA can also prevent GVHD without the need for global immunosuppression [181]. LAG-3 also serves similar inhibitory functions and is expressed on the surface of CD4 [182] and CD8 [183] T cells. Preferential expansion of LAG3⁺ T cells has been observed in patients with cancer and such cells secrete immunosuppressive cytokines like IL-10 and TGF-β1, but not IL-2 [182]. Combined blockade of LAG-3 and PD-1 could restore normal proliferation and cytokine production in CD8 T cells [183]. The KLRG1 receptor is unique in a way that it is an NK cell surface receptor also found on T cells. Until recently, it was thought to be a marker for T cell memory and senescence, but more recent research has indicated an inhibitory role for this cadherin receptor. KLRG1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) motif which is used in inhibitory signaling to bring down the proliferative and cytokine signaling abilities of T cells [184].

A global representation of all the negative regulators has been presented by Bengsch and colleagues where they have reported co-expression of 2B4, CD160 and KLRG1 along with PD-1 in 50% of the CTLs from chronically HCV infected patients. Consistent with the individual outcome of each regulator, the CD8 T cells with an up-regulated co-expression of these markers were low in proliferative capacity, showed variations in sequences of corresponding epitopes and possessed a diminished T cell differentiation stage. Conversely, the cells with a downregulated co-expression of the above markers showed proficient T cell activity [185].

Thus, the presence of negative immunoregulatory receptors and absence of costimulatory factors may have pleiotropic effects on T cell function and apoptosis. This is in line with the two-signal hypothesis that proposes that tumours without co-stimulatory signals not only fail to induce CTLs but delete them [186,187]. A fibrosarcoma lacking co-stimulatory signals

however did not delete CTLs but rather primed them directly if tumour cells reached secondary lymphoid tissues early [188,189]. These results were termed as an exception because antigen-presenting cells of the host may have reprocessed tumour antigen onto MHC class I molecules (termed 'cross-priming' or 'cross-presentation') [190]. Results of Ochsenbein *et al* also document that neither B7⁺ nor B7⁻ strictly extra-lymphatic tumours can induce or delete T cells, negating predictions of the two-signal hypothesis [191]. Therefore, T cell deletion by tumours is probably not through negative signals but more likely a result of 'over-induction' or 'exhaustion', suggested for CD4⁺ T cells [192] or for CD8⁺ CTLs [191,193]. More work is required to understand the intricate balance between positive and negative immuno-regulatory receptors in clinical tumours.

Various immune checkpoint therapies are currently being developed to overcome the negative signals mediated by tumour cells. Clinical trials to block the inhibitory signaling of CTLA-4 by antagonist antibodies in melanoma showed some success [194,195], following which clinical trials with other immune checkpoint strategies focused on PD-1 and PD-L1 in melanoma as well as renal and lung carcinomas, the cancers so far most responsive to immunotherapy. These clinical trials are summarized in Table 3. CTLA-4 antibody, ipilimumab, was approved by the United States Food and Drug Administration (US FDA) for the treatment of melanoma in the year 2011, followed by PD-1 antibodies, pembrolizumab and nivolumab, for melanoma as well as non-small cell lung cancer (NSCLC) during 2014–2015. The PD-1 antibody, pembrolizumab, showed a 19.4% response rate in patients with NSCLC, and was granted a “breakthrough therapy” designation for the treatment of NSCLC. Response rates upon pembrolizumab treatment significantly improved to 40% when the tumours expressed high levels of the ligand PD-L1 suggesting that the expression of PD-L1 on tumour cells may act as a predictive immune biomarker [196]. PD-1 antibody, nivolumab, was approved on fast track to treat patients with advanced metastatic squamous NSCLC with progression on or after platinum-based chemotherapy [197]. To further improve the therapeutic efficacy of immune checkpoint inhibition, these strategies are also being tested actively in combination with chemotherapy or targeted therapies in locally advanced or metastatic cancer patients.

The immune checkpoint PD-1 antibodies have shown superior responses over CTLA-4 antibodies, most likely because the PD-1 pathway blockade acts locally in the tumour microenvironment. Also, the response of tested cancers to immune checkpoint inhibition appears to be independent of driver mutations present in most cancers. However, this needs to be investigated more carefully as mutational load in cancer cells may give rise to neoepitopes that may facilitate immune recognition by effector lymphocytes. Indeed, in a recent study a higher nonsynonymous mutation burden in NSCLC patients treated with pembrolizumab correlated with the higher neoantigen repertoire, which was associated with improved objective response, durable clinical benefit, and progression-free survival in one responder [198]. Thus, the genomic landscape of cancers may shape response to the immune checkpoint therapy. However, it should be borne in mind that the PD-1 inhibitors result in an objective durable response only in a minority of patients. Further research is needed to find a reliable biomarker of response, in order to guarantee that only patients who will respond receive treatment. In addition, due to the dynamic nature of immune response to tumours

and the complexity of the multiple immune checkpoint pathways, it will be important to identify synergistic combinatorial regimens that could target a broad spectrum of cancer cells and overcome other hurdles presented here-in-after.

5. T cell ignorance/ accessibility of tumour

Early trapping of tumour cells in secondary lymphoid organs— lymph nodes and spleen: the main places where T cells await activation — is beneficial as it induces CTLs to reject tiny tumours early, at a time when selection of immune evasion mutants has not yet taken place. Once a tumour has reached a certain size and effector CTLs are induced late, the outcome reflects a race of growing tumour masses and limited effector cells versus tumour mutant selection. Some metastasizing tumours that fail to 'seed' lymph nodes and spleen are 'ignored' by the immune system [193]. When tumours are injected into secondary lymphoid organs, they segregate into two types [191]. One type circulates in lymphoid regions, intermingling with T cells and allowing direct antigen presentation to T cells and activation of antitumour immunity. These tumours are considered 'immunogenic' and elicit a strong immune response. Another type of tumours that are weakly immunogenic rely on the indirect pathway of antigen cross-presentation via dendritic cells. Even if this type of tumour metastasizes to the lymph nodes, it grows as nodules, 'walled off' by matrix barriers of haemostasis factor or collagen from the T cells, which therefore remain ignorant of the tumour antigens [191]. Similar indications were observed in a setting in which model tumour EL4 rejection was solely dependent on tumour-specific CD8⁺ T cells [25]. Small immunogenic tumours failed to induce a rejection response, despite the fact that they were not ignored: tumour-specific CD8⁺ T cells became activated differentiated into effector cells and infiltrated the tumour bed. Nevertheless, tumour rejection did not occur. In sharp contrast, the same immunogenic tumour, when growing as a large tumour mass, was rejected by transferred tumour-specific CD8⁺ T cells. The main features which distinguished the rejection response to a large tumour mass from the response to a small tumour was that, in the latter case, activated CD8⁺ T cells appeared much later, and in much smaller numbers. Efficacy of adoptive transfer was thus dictated by the size of the tumour mass at the time of transfer.

Another type of cells that can contribute to the accessibility of a tumour are the $\gamma\delta$ T cells. These cells possess a unique property of antigen presentation to T cells in a non-MHC dependent manner and are now being investigated as immunotherapeutic agents. Aminobisphosphonate compounds known for their antiangiogenic, antiosteolytic and pro-apoptotic properties, have been successfully employed for stimulating $\gamma\delta$ T cells in vitro [199–201]. In multiple myeloma patients, zoledronic acid could stimulate DCs to activate autologous V γ 9V δ 2 T cells [202]. Phase I clinical trial with V γ 9V δ 2 T cells, expanded in vitro in culture, yielded successful results but only in combination with other therapies [203].

Thus, the ultimate anti-tumour immune response may depend on where the tumour-specific antigens are present, how they are presented and when the responsive antigen-specific T cells reach the site of tumour. Tumour invasion and angiogenesis across normal tissue and metastasis may disrupt the intercellular matrix generating pro-inflammatory signals that

prepare the host immune system to respond. If the tumour does not generate or present immune responsive antigens during the pro-inflammatory phases of its development, then immune ignorance may ensue. But if the tumour allows presentation of a pool of immunogenic antigens just when it is generating a pro-inflammatory response, the outcome will be a potent anti-tumour response. These tumours will be eliminated naturally unless they have developed specific ways of resisting immune recognition, such as downregulation of MHC or the antigen-processing machinery.

6. Regulatory T cells and their role in cancer

Regulatory T cells that express the forkhead type transcription factor “Foxp3” is essential for normal immune function [204–206]. Absence of T_{Reg} cells results in multi-organ autoimmunity and death. The CD4⁺CD25⁺ Foxp3⁺ T_{Reg} cells develop in the thymus, represent 5–10% of CD4⁺ T cells in the periphery, and are distinguished by their specific and universal expression of the Foxp3, which serves as a control factor for their development and function [207,208]. The T_{Reg} cells acquire suppressive potential in vitro in response to TCR and TGF- β stimuli [209,210]. TGF- β , as discussed earlier, is a pluripotent cytokine that has pronounced effects on T cell-mediated immune suppression as well as on the control of autoimmunity [209,211,212]. In cancer, TGF- β signaling regulates tumour initiation, progression and metastasis through a diverse repertoire of tumour-cell-autonomous and host-tumour interactions, and also helps in the conversion of naïve CD4⁺ T cells to T_{Reg} cells that express Foxp3 [206,210,213]. This causes an increase in the number of T_{Reg} cells and a decrease in CD8⁺ T cells at tumour site in breast, gastric and ovarian cancer [214]. There are several reports suggesting the increase in number of T_{Reg} cells at the tumour site. It is possible that proliferation and death of tumour cells may present a source of self-antigens, which T_{Reg} cells may identify and gets recruited at tumour site [215]. Another mechanism suggests that macrophages at tumour site produce chemokine CCL22 which chemo-attracts CCR4 expressing T_{Reg} cells [216,217]. An inflammation at tumour site also plays significant role in increasing the number of T_{Reg} cells at tumour site [218,219]. The presence of increased numbers of T_{Reg} cells in cancer suppresses an effective anti-tumour immune response [213,220,221]. Hence, a clear understanding of role of T_{Reg} cells during tumour progression and clearance is needed to develop a targeted therapy to reduce number of T_{Reg} cells in tumour microenvironment.

T_{Reg} cells suppress self or autoreactive cells, thereby protecting the host from autoimmune diseases. On the other hand, T_{Reg} cells may block anti-tumour immune responses. Particularly in the context of cancer, T_{Reg} frequency and function are important because increased numbers might favor tumour development or growth and influence the course of the disease [206,220,222,223]. The development of T_{Reg} cells during tumour progression has been addressed in several model systems. For example, T_{Reg} development in a fibrosarcoma model in C57BL/6N mice as well as in a colon adenocarcinoma model in BALB/c mice has been shown to have lethal tumour progression [147]. The T_{Reg} cells during tumour development have been shown to have high suppressor capacity. The suppressive effect of naturally occurring T_{Reg} cells against tumour-specific CD8⁺ T cells was established in a poorly immunogenic B16 melanoma model [224]. In this model, T_{Reg} cells efficiently suppressed CD8⁺ T cell –mediated immunity. These findings clearly suggest

that T_{Reg} cells are major regulators of tumour immunity [224]. Further evidence for the interference of T_{Reg} cells with CD8⁺ T cell-mediated anti-tumour immune response in vivo was established in a transgenic murine colon carcinoma model, where T_{Reg} cells abrogated CD8⁺ T cell-mediated tumour rejection by specifically suppressing CTLs [213].

Commonly, the T_{Reg} cells hamper the anti-tumour immunity and work as an obstruction in immunotherapy [225]. In recent years, several strategies have been developed to target T_{Reg} cells population by depletion, inhibition of function, and modulation of T cell plasticity. It has been shown that depletion of T_{Reg} cells by administration of anti-CD25 monoclonal antibody (mAb) inhibits tumour growth in different models [226,227] but the global depletion of T_{Reg} cells causes other problems such as development of autoimmune and inflammatory symptoms. Some molecules that directly or indirectly control the T_{Reg} cell function are Toll like receptor (TLR), CTLA-4, Glucocorticoid-induced TNF receptor (GITR) and folate receptor (FR). TLR signals (lipopolysaccharide [LPS] or CpG) block the suppressive activity of T_{Reg} cells partially by IL-6-dependent mechanisms [228]. GITR is a co-stimulatory molecule present on resting CD4⁺ and CD8⁺ T cells and expressed by T cell activation. This molecule is highly expressed on T_{Reg} cells. Treatments of T_{Reg} cells with anti-GITR mAb have shown to inhibit the suppressive activity of T_{Reg} cells [229–231]. CTLA-4 is upregulated upon TCR activation and is expressed on T_{Reg} cells. Inhibition of CTLA-4 has shown to decrease the suppressive activity of T_{Reg} cells [232,233]. In rodents, T_{Reg} cells express a higher level of FR4 and serve as a marker, which differentiate between activated T_{Reg} cells and activated effector T cells [234]. The anti-FR4 depleting mAb depletes activated T_{Reg} cells while preserves tumour-reactive effector T cells [234].

Availability of reagents for T_{Reg} depletion in animal models generated interest in developing strategies to target T_{Reg} cells during cancer. For instance, low-dose cyclophosphamide treatment selectively reduces or inhibits the T_{Reg} cell population [235]. In a recent study, the role of cyclophosphamide in combination with bacillus Calmette-Guerin (BCG) on T_{Reg} cells has been tested suggesting a dose dependent decrease in the number of T_{Reg} cells. Thus, a dose titration strategy to control T_{Reg} population during tumour development and progression could be used as an alternate approach. Several studies [236–240] using Denileukin diftitox, a fusion protein of IL-2 and diphtheria toxin that targets CD25-expressing cells, have reported reduction of the percentage of T_{Reg} cells in the peripheral blood of patients with ovarian cancer, renal carcinoma, and melanoma. The anti CD25 treatment resulted in the depletion of number and percentage of T_{Reg} cells in tumour [241]. Another study has reported that the use of anti CTLA-4 antibodies alone or in combination with peptide vaccination improved survival of patients with melanoma by promoting antitumour response (by increasing T effector activation and may be by reducing T_{Reg} population, not fully defined yet) [242,243]. As mentioned above, GITR is constitutively expressed on T_{Reg} cells. The anti GITR antibody, DTA-1, reduces the suppressive capacity of T_{Reg} cells and activates the effector function of T cells [244–247]. The potential use of GITR in the regulation of T_{Reg} cell function needs a careful investigation.

In general, all the above-mentioned intervention approaches have shown a reduction in immunosuppressive functions of T_{Reg}, thereby allowing an increase in numbers of NK and cytotoxic T cells. On the other hand, any global solution or treatment of T_{Reg} cell that

deplete or inhibit the functions of T_{Reg} cells enhances the chance of developing autoimmunity. Therefore, a careful approach is needed to target T_{Reg} cell population and at the same time needs to maintain a balanced ratio of number of T_{Reg} to effector T cells in tumour microenvironment. The targeted depletion of T_{Reg} cells would be best option to minimize the risk of developing autoimmunity. In the current scenario, inhibiting the function of T_{Reg} cells at tumour level should be a better strategy because it will allow preferential inhibition of T_{Reg} function.

A rare subset of T_{Reg} cells, the $\alpha\beta$ TCR⁺CD3⁺CD4⁻CD8⁻NK1.1/CD56⁻ double-negative (DN) T_{Reg} cells, functions in a uniquely different manner as it can inhibit graft-versus-host disease (GVHD) mediated by CD8⁺ T cells as shown in a 2C transgenic mouse model [248]. This subset was initially demonstrated to be active in inducing tolerance to allogeneic skin grafts by cytolysis of allo-specific T cells [249]. Such DN cells have also been reported to induce apoptosis and suppress antigen-specific CD8⁺ T cells in humans [250]. This relatively novel subset of T_{Reg} cells can help promote tolerance by negative regulation of the immune response. This particular subset could be expanded in contrast to the general T_{Reg} cells discussed above, in order to minimize GVHD to achieve greater tolerance to immune transplants during immunotherapy.

7. Cancer immunoediting by T cells

The cancer immunosurveillance theory originally formulated in 1957 by Burnet and Thomas [251], states that lymphocytes survey the body for specific antigens expressed by newly arising tumours. This theory has been revalidated by recent work. Cancer incidence in the RAG^{-/-} and/or STAT-1^{-/-} mice is higher than in normal mice [252]. RAG^{-/-} mice largely suffer from cancer of epithelial tissue in the intestine. Double RAG^{-/-}STAT-1^{-/-} develop analogous breast cancers as well. Both types of mice particularly develop spontaneous tumours at an age of around one year and beyond. Conversely, engineered activation of STAT5 transcription factors bestows high efficiency and prolonged antigen responsiveness in effector T cells by amplifying the tumour homing capability of endogenous T cells along with upregulation of granzyme B expression [49,253]. In another study, host IFN- γ and direct activity of cytotoxic lymphocytes expressing perforin independently contributed to antitumour effector functions that together control the initiation, growth, and spread of experimental and spontaneous tumours in mice [254]. So, even with important elements of the adaptive immune system missing, the mice can survive for a considerably long period most likely by the components of the innate immunity. This suggests that the role of immune system in limiting tumourigenesis is more subtle than implied by the classical immuno-surveillance theory. Studies in the last decade have suggested that immunosurveillance primarily functions as a component of a more general process of cancer immunoediting [252,254–257]. Tumour cell variants which have survived the elimination phase intrinsic to the immunosurveillance theory enter the equilibrium phase. In this phase, lymphocytes and IFN- γ exert a selection pressure on tumour cells which are genetically unstable and rapidly mutating. Tumour cell variants which have acquired resistance to elimination then enter the escape phase. In this phase, tumour cells continue to grow and expand in an uncontrolled manner developing into full-blown malignancies.

Thus, a modified version of immune surveillance has emerged in which lymphocytes and IFN- α , β and γ collaborate to act as an 'extrinsic suppressor' of tumours in certain locations or tissues [252,258]. This is analogous to the classic intrinsic suppressors, such as that encoded by the *p53* gene, which must be inactivated or bypassed to allow tumour progression. Further, tumours that develop in the presence of an intact immune system are less immunogenic than those that develop in immunodeficient hosts [252,259]. Thus, tumours are imprinted by the immunologic environment in which they form. These protective and sculpting actions of the immune response on developing tumours were termed 'cancer immunoediting'. The scope of this process of 'cancer immunoediting' is broad, with a potential to (a) promote complete elimination of some tumours, (b) generate a non-protective immune state to others, or (c) favour the development of immunologic anergy/tolerance/indifference, and (d) favour an immune-equilibrium whereby in immunogenic tumours, tumour-specific adaptive immune response contributes to not only the development of tumour escape variants but also the maintenance of the "occult" cancer in the equilibrium state [256,257].

8. Role of Gene Engineering in T cell therapy

Effective T cell immunotherapy depends on efficient and timely recognition of tumours by T cells. However, tumours are well known to alter or completely eliminate class-I MHC molecules from their surface in order to escape T cell recognition. This brought into focus, the development of a T cell which did not need antigen presentation mediated by the HLA molecule, thus leading to the development of chimeric antigen receptors (CARs) and T cells engineered to express such receptors on their surface. T cells are engineered such that a single chain variable fragment (scFv) is expressed on the extracellular domain while being fused to the CD3 ζ chain in the cytoplasm for signaling functions [260–263]. This strategy helps bypass the need for an HLA molecule for T cell activation. CAR expressing T cells have been tested and also put to clinical trials [264–269]. More recently, two simultaneous studies have reported greater than 1000 fold expansion of T cells expressing CARs after infusion in patients with advanced chronic lymphocytic leukemia (CLL). The CARs were designed with specificity for the B-cell antigen CD19 and were conjugated with CD137 of the T cells. Expression of CARs was observed up to six months and eliminated approximately 1000 CLL cells. Few cells persisted as memory CAR⁺T cells after complete remission was observed in patients [270,271]. The use of CAR-engineered T cells in adoptive immunotherapy has been reviewed by Liu and co-workers [272]. In a recent study, the efficiency of two different vectors was compared in terms of efficient transduction of murine T cells and strong gene-transfer capabilities. The γ -retroviral vectors are found to be more efficient than lentiviral vectors for such a transduction [273].

While genetic engineering of human hematopoietic stem cells (hHSC) has led to the development of CD8 T cells which can undergo appropriate selection in the thymus. Attempts have been made to amplify T cell populations in vitro while genetically modifying them ex vivo before being infused into the patient as part of adoptive T cell therapy. The hHSCs were genetically modified in a human/mouse chimera and could generate significant CD8 T cell population that could abrogate melanoma tumours in vivo [274], while "reprogramming of T cells" [275] modifies them to adopt a desired effector function when

specific cytokines are added to the culture medium [276,277]. In a study by Stephan and colleagues [278], adjuvant drug-loaded nanoparticles were conjugated to the surface of the T cells in order to enable T cells to effectively reach their target. These approaches can help overcome limitations like reduction in viability and function of the transplanted cells by a reduction in side effects.

The term “Bionic cell” was introduced by Pardoll [279] while recapitulating the concept of auto-costimulation in T cells explored by Stephan and colleagues [280]. This group engineered T cells to express a chimeric single-chain antibody which was specific to the tumour antigen. This antibody was linked to signaling components of the chain and the cells were also genetically modified to express CD80 and 4-1BBL. Upon encountering a tumour cell, the antibody recognizes the tumour antigen, while the costimulatory molecules on the surface of the same cell help in auto-costimulation, thus enhancing tumour abrogation by the T cell. In a recent effort to eradicate cancer stem cells in pancreatic cancer, Cioffi and colleagues succeeded in “redirecting” CD8 T cells against tumour by the use of a bi-specific antibody, MT110 [281].

9. Future prospects

9.1. Circumstances under which T cell therapy can destroy large tumour burden

T cell immunotherapy against cancer faces several hurdles as illustrated in Fig 1. These hurdles together restrict the anti-tumour cytolytic efficacy of T cells. These fall into the following broad categories: (a) self-nature of most tumour antigens so that the TCR repertoire reactive to the peptide ligands derived from these tumour antigens have mostly moderate/low affinity; (b) low levels of costimulation; (c) active suppression of incipient “self” responses elicited by tumour antigens by different suppressor cells, such as CD25⁺CD4⁺ T_{Reg} cells, $\gamma\delta$ T cells, $\alpha\beta^{\text{int}}$ T cells, IL-13 secreting NKT cells, CD8⁺ T suppressor cells and CD11b⁺Gr-1⁺ myeloid suppressor cells; or (d) active tolerance, anergy, or deletion of T cells, due to lack of CD4⁺ T cell help in some tumours or exhaustion following T cell overstimulation. Thus, in order to have an effective antitumour T cell response, one would require (a) an optimal level of T cell activation by using altered peptides or novel antigens [282] (b) persistence of tumour-specific T cells in high numbers that could be achieved by providing γc homeostatic cytokines such as IL-7, IL-15 and IL-21, (c) blocking of suppressor factors/cells, (d) availability of CD4⁺ T cell help, and (e) avoidance of overstimulation. Conditions that may favor the success of T cell immunotherapy of tumour are summarized in Table 4.

9.2. Mechanisms of tumour destruction used by T cells in vivo

Another relevant issue is to understand what mechanisms a T cell employs to eliminate tumour cells. Tumour destruction may be orchestrated by CD8⁺ T cells *in vivo* by three main mechanisms: (1) Direct cell-mediated cytotoxicity with infiltrating CD8⁺ T cells destroying tumour cells; (2) Local inflammation, and the activated CD8⁺ T cells, resulting in an accumulation of other innate immune effector cells that alone or together with CD8⁺ T cells participate in direct tumour destruction; and (3) Production by the CD8⁺ T cells of factors

that alter the local tumour microenvironment, such as TNF- α and IFN- γ , affecting tumour stromal cells in a way that is detrimental to the tumour.

Based on data generated by 4–20 h *in vitro* cytotoxicity assays with a number of tumour models, most of the specific tumour lysis by CD8⁺ T cells can be accounted for by the exocytosis of lytic granules from the effector cells or by the production of “death ligands” of the TNF superfamily (particularly FasL or TNF) that promote tumour cell apoptosis. It has been reported that direct T cell-mediated tumour regression and long-term antitumour immunity are perforin (Pfp) and FasL independent [283]. TRAIL has also been shown to be capable of limiting the development of metastases [284]. However, at least in mice, TRAIL does not seem to be a major product of activated CD8⁺ T cells. This suggests that any contribution of TRAIL to tumour destruction *in vivo* may involve recruitment of cells of the innate immune system such as NK cells [285]. A close collaboration of CD8⁺ T cell and NK cells has been demonstrated for effective antitumour response [259,286]. Experiments performed in mice injected with renal cell carcinoma Renca cells that express viral hemagglutinin (HA) as a surrogate tumour antigen suggest that FasL expression on T cells is a crucial factor in limiting the establishment of the Renca-HA tumours even under the situations when the tumour cells express very low concentrations of HA antigen [287]. It remains to be seen if the FasL expression on innate NK cells or effector cytokines alone secreted by immune effector cells, are equally important in eradicating tumour cells *in vivo*.

10. Conclusions

The generation of immune response against tumours is a multivariable process requiring the actions of different immune effectors in a manner dependent on the tumour's cell type of origin, mechanism of transformation, anatomic localization, and mechanisms of immunologic recognition and checkpoint regulation. This complex process requires a concerted effort of the various cells of the adaptive immunity in combination with innate immune cells. A consistent observation from a number of studies is that the T cell inhibition in the tumour bearing host, if any, is not permanent and can be reversed. The T cell inhibition largely observed in the tumour-bearing hosts particularly in the situations of the large tumour burden is rather a bystander effect of the tumour growth-associated chronic inflammation, oxidative stress and immunosuppressive tumour microenvironment. Encouraging studies are present that demonstrate the ability of adoptively transferred antitumour T cells to mediate the rejection of tumours in mice under appropriate conditions of host immunostimulation. Increasing knowledge of how tumour is destroyed *in vivo* should allow for the design of effective antitumour therapeutic protocols in combination with (a) the targeted drugs which minimize tumour burden prior to immunotherapy without immunosuppression, or (b) sensitizing drugs (e.g. bortezomib, triterpenoids) which enhance the tumour's sensitivity to directly administered death ligand proteins or agonist antibodies [288,289], or immune cells providing a natural source of death ligands [290], or reduce tumour resistance to effector CD8⁺ T cells following adoptive transfer. Furthermore, a close collaboration of the innate and adoptive components of the immune system should be emphasized in view of the more recent work in different immune conditions [291] showing their interdependent synergy for an optimal anti-tumour immune response. An optimal clinical protocol should attempt to combine those strategies with complementary

immunostimulatory schemes, which can overcome the tumour-associated immunosuppression, such as stimulation of Notch signalling in lymphopoietic cells by immunopotentiating Notch ligand formulations [107,292,293]. Future efforts should therefore be directed at deciphering the close regulatory links between the adaptive effector mechanisms and the innate components of the host that can trigger the sufficient and continued activation of the anti-tumour effectors, in combination with other less specific immunostimulatory and tumour-sensitizing therapeutic drugs.

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Highlights

- A cancer evades the host immune response to ensure its development and survival.
- Concerted actions of various immune cells determine an anti-tumour immune response.
- Immunosuppressive tumour microenvironment presents a challenge for T cell response.
- Hurdles that the adoptive T cell immunotherapy against cancer faces are summarized.
- Checkpoint conditions for effective eradication of large tumour burden are discussed.

Figure 1. Major hurdles faced by T cells in tumour microenvironment

1. Tumours alter their surface expression of HLA and MHC molecules which prevent T cells from binding to tumour. Tumour cells sometimes either lack co-stimulatory molecules and/or overexpress inhibitory molecules, which help them escape T cell recognition and negatively influence T cell function. 2. Tumour growth causes abnormal TCR-CD3 complex formation which results in an unproductive signal transduction after TCR: MHC-peptide ligation. Tumour growth decreases the dissociation of TCR:MHC complex which leads to inefficient recognition of tumour antigens by cytotoxic T lymphocytes (CTL) leading to reduced lysis of tumour cells. Immunosuppressive gangliosides expressed on tumour cells

act to decrease signal transduction by T cells leading to tumour motility and invasiveness. Indoleamine 2,3-dioxygenase (IDO) catabolises tryptophan (Trp) whose deprivation in turn leads to T cell apoptosis and impaired tumour rejection. 3. If a tumour is exposed to the T cell in the early stages of its formation, it can be efficiently eliminated by the T cells. If the tumour reaches an advanced “walled off” state, the T cells ignore tumour cells resulting in tumour escape and metastasis. 4. Tumour cell variants develop to escape the immunosurveillance carried out by T lymphocytes. Such variants develop under the pressure of an active antigen-specific T cell response. These tumour cells escape immune elimination and maintain equilibrium within the host. 5. The tumour microenvironment induces the immune cells to change their redox potential and secrete reactive oxygen and nitrogen metabolites and angiogenic factors. Tumour-induced suppressor T cells produce immunosuppressive cytokines which inhibit IL-12 production and increase IL-10, IL-6 and IL-13 expression. Tumour growth inhibits myelopoiesis resulting in an immune imbalance caused by accumulation of immature myeloid cells at the tumour site favouring tumour progression.

Table 1**Modulation of T cell functions as a result of tumourigenesis**

Lack of antigen processing, T cell recognition, and TCR signaling	
-	Decreased levels of surface CD3 ζ chain, phospholipase C γ 1, p ^{56lck} , p ^{59fyn} and tyrosine phosphorylation of ZAP-70 on T cells
-	Reduction in NF- κ B/Rel family of transcription factors
-	Loss of HLA or MHC class I molecules on the tumour
-	Loss of β 2m and TAP molecules and downregulation of antigen processing
-	Blockade of T cells by negative immunoregulatory receptors, such as PDL-1 expressed on tumour cells
-	NK-like inhibitory receptor / ligand interactions
Negative immune modulation	
-	Immune cell-production of suppressive cytokines TGF β , IL-10, and IL-13
-	Production of TGF β , IL-10, prostaglandins, VEGF and FasL by tumour cells
-	Immune imbalance by an increase in immature macrophages, DCs, and myeloid cells and immunosuppressive T _{Reg} and NKT cells
-	Tumour production of arginase and reactive oxygen and nitrogen intermediates
-	Tryptophan degradation by tumour expression of IDO
-	Shedding of gangliosides by tumour
-	Enhanced T cell apoptosis
-	Lack of common γ -chain survival cytokines
Non-accessibility of tumour to T cells	
-	Tumour stromal cell entrapping of T cell effectors
-	Barriers of haemostasis factor or collagen around tumour
-	Lack of sufficient chemokine expression by tumour or myeloid cells at the site of tumour
Immune editing, equilibrium and escape	
-	Selection of immunorefractory tumour cell variants during antitumour immune response
-	Co-existence of tumour cells in immune equilibrium
-	Development of immune escape e.g. antigen loss tumour cell variants

Table 2

Studies that suggest T cell dysfunction in tumours

Tumour Type	T Cell Defect	References
Murine <ul style="list-style-type: none"> Colon Renal cell carcinoma Fibrosarcoma 	<ul style="list-style-type: none"> Decreased steady-state levels of mRNA and protein expression for $p^{56\text{lcck}}$ and $p^{59\text{fyn}}$ Oxidative stress by tumour-associated macrophages inducing structural changes in $CD3\gamma\delta\epsilon$ Decreased TCR-associated PTK activity and failure of ZAP-70 to associate ζ chain Reduced levels and abnormalities of the NF-κB/Rel family of transcription factors 	[30,31,42,131,134,294,295]
Human <ul style="list-style-type: none"> Renal cell carcinoma Colorectal carcinoma Hepatic carcinoma Head and neck squamous cell carcinoma Melanoma 	<ul style="list-style-type: none"> Decreased level of $CD3\zeta$ chain and $p^{56\text{lcck}}$ Abnormal assembly of the TCR-CD3 complex Abnormal pattern of NF-κB-specific DNA-binding activity Decreased protein tyrosine phosphorylation and <i>in vitro</i> kinase activity of $p^{56\text{lcck}}$, in spite of normal levels of the $CD3\zeta p^{56\text{lcck}}$, $p^{59\text{fyn}}$ and ZAP-70 	[33–35,41,295]

Table 3

Use of immune checkpoint inhibitors in clinical trials.

Checkpoint Blockade	Antibody	Trial Status	Clinical Trial
Cytotoxic T lymphocyte antigen-4 (CTLA-4)	Ipilimumab (Yervoy)	Approved by the US FDA in 2011 for use in melanoma. Undergoing phase II trial in stage IV melanoma	NCT02054520
		Phase II trial in combination with nivolumab or bevacizumab for metastatic renal cell carcinoma	NCT02210117
		Phase III trials for Non-small cell lung cancer (NSCLC) and Small cell lung cancer (SCLC)	NCT01285609 NCT01450761
	Tremelimumab	Phase I trial in combination with MEDI4736 in melanoma Phase I trial for renal carcinoma Phase II trial for mesothelioma	NCT02141542 NCT01975831 NCT01655888
Programmed cell death-1 receptor (PD-1)	Pembrolizumab (Keytruda)	Approved by the US FDA in September 2014 for use in melanoma. Undergoing phase II trial for unresectable stage III and unresectable stage IV Melanoma	NCT02306850
		Phase I/III trial in advanced renal cell carcinoma and urothelial cancer	NCT02133742 NCT02256436
		Approved by the US FDA in September 2014 and granted a "Breakthrough Therapy" designation for the treatment of NSCLC	NCT01876511 NCT01905657 NCT02085070 NCT02054806 NCT00730639
	Nivolumab (Opdivo)	Approved by the US FDA in December 2014 for melanoma. Undergoing phase III trial in prevention of recurrence of melanoma after complete resection of stage IIIB/c or stage IV melanoma Phase III trial in combination with ipilimumab (Yervoy) for untreated advanced or metastatic renal cell Carcinoma Approved by the US FDA in March 2015 for the treatment of squamous NSCLC that has failed chemotherapy	NCT02388906 NCT02231749 NCT02066636
PD-1 ligand (PD-L1)/B7 homolog 1 (B7-H1)	MPDL3280A	Phase I trial in melanoma	NCT02154490
		Phase II trial in advanced renal cell carcinoma	NCT01984242
		Phase I/II trial for locally advanced or metastatic NSCLC	NCT01846416 NCT02031458 NCT01375842
	MEDI4736	Phase I/II trial for melanoma Phase I trial for renal carcinoma Phase II/III trial for locally advanced or metastatic NSCLC, or second-line therapy in recurrent stage IIIB-IV NSCLC	NCT02027961 NCT01975831 NCT01656642 NCT02087423 NCT01693562 NCT02088112

Table 4

Conditions that favour the success of T cell immunotherapy against cancer.

Sustained high levels of tumour-specific T cell activation
Increased IL-2, IL-7, IL-15, and IL-21
Altered peptide ligands
CD4 T helper cell conditioning
Early trapping of tumour cells in the secondary lymphoid organs
Persistence of high numbers of tumour specific T cells by abundance of survival cytokines
Avoidance of immune-survival cytokine sinks
Blocking of suppressive factors/ suppressor cells
Blocking the disruption of intercellular matrix by tumour angiogenesis
Increased innate adjuvants such as NKG2D ligands, TLR ligands, and CpG
Enhanced intrathymic T cell production
Tumour cell sensitization to apoptosis and FasL-mediated cytotoxicity
Improved genetically engineered T cells
Enhanced collaboration between adaptive and innate effector cells
Enhanced hematopoietic Notch signaling for anti-tumour lymphocyte repertoire and function
Avoidance of chronic inflammation and T cell tolerance
Immune checkpoint inhibition