

How tumors might withstand $\gamma\delta$ T-cell attack

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Abstract Several clinical trials are currently assessing the therapeutic activity of human TCRV γ 9V δ 2⁺ lymphocytes in cancer. Growing tumors usually follow a triphasic “Elimination, Equilibrium, Escape” evolution in patients. Thus, at diagnostic, most tumors have already developed some means to escape to immune protection. We review here the conventional immunescape mechanisms which might also protect against cytolytic TCRV γ 9V δ 2⁺ lymphocytes activated by phosphoantigens. Neutralization of these deleterious processes might prove highly valuable to improve the efficacy of ongoing $\gamma\delta$ cell-based cancer immunotherapies.

Keywords Cancer · Gamma delta lymphocytes · Immunescape · Immunotherapy

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Introduction

The antigen specificity and function of TCRV γ 9V δ 2⁺ human $\gamma\delta$ T lymphocytes as well as clinical trials assessing their anticancer activity have been described in other articles in this review. We therefore focus here on the means by which the tumor and its niche attempt to protect against immune attack by these cells.

Reducing the recruitment of tumor infiltrating lymphocytes (TIL) by tumor vasculature defects represents a first and straightforward—though relatively unspecific—line of defense against the TCRV γ 9V δ 2⁺ cells. Once these lymphocytes have solved this first issue and successfully reached the tumor niche, various resident cells of the tumor stroma exert potent immunosuppressive activities able to reduce the $\gamma\delta$ cell functions. Then, various soluble factors that are possibly released from this stroma or directly by the tumor cells themselves represent a highly efficient means to abrogate immune responses (Fig. 1).

These successive levels of immune escape mechanisms are discussed below.

Tumor infiltration by TCRV γ 9V δ 2⁺ T lymphocytes

The direct intra-tumoral injection of TCRV γ 9V δ 2⁺ T cells reduces various tumor xenografts, suggesting that these cells are efficient when reaching the tumor site [1, 2]. So far, however, no clinical trials with either ex vivo expanded $\gamma\delta$ T cells or ABP injection have monitored the tumor infiltration by TCRV γ 9V δ 2⁺ cells [3–5]. In fact, although $\gamma\delta$ T cells are frequently found among TILs, only a minority of these cells are from the TCRV γ 9V δ 2⁺ subset [6, 7]. Therefore, the limited efficacy of anti-tumoral

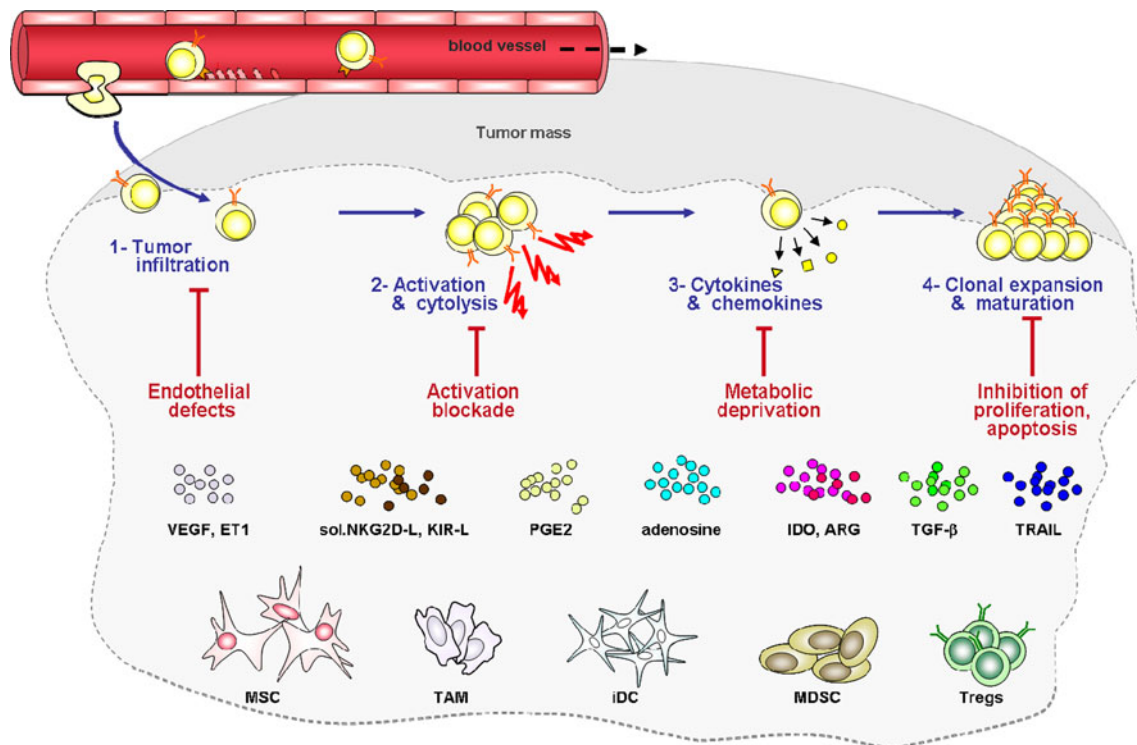


Fig. 1 TCRV γ 9V δ 2⁺ cell functions and immunoevasion pathways in tumors

TCRV γ 9V δ 2⁺ cell may just reflect their inability to infiltrate established tumors.

Leukocyte extravasation is tightly controlled by the endothelium through multiple integrins and vascular selectins. Lymphocyte adhesion to the endothelium is usually weak in tumor microvessels [8]. Endothelial cells from human tumors express very low levels of critical molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). In addition, the tumor endothelium prevents T cell infiltration upon over-expression of the endothelin B receptor (ET_BR). Endothelin/ET_BR signaling inhibits ICAM-1 expression on human endothelium and ET_BR neutralization in mice favors TIL recruitment and cancer immunotherapy [9].

Leukocytes migration from peripheral blood to the tumor is directed by chemokines that regulate the endothelial adhesion of leukocytes. Unfortunately however, the chemokine expression profile of most human cancers is far more prone to recruit immunosuppressive cells than useful lymphocytes [10, 11]. The high production of CXCL12 by cancer-associated fibroblasts [12] could nevertheless promote TCRV γ 9V δ 2⁺ T cells infiltration, as this chemokine regulates the migration of TCRV γ 9V δ 2⁺ T lymphocytes to renal carcinoma cell lines [13]. CXCR4 is expressed by peripheral blood TCRV γ 9V δ 2⁺ T cells and transiently increased after phosphoantigen stimulation [14].

Nevertheless, peripheral blood TCRV γ 9V δ 2⁺ T cells predominantly express CXCR3 and CCR5 chemokine receptors for the pro-inflammatory chemokines CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (ITAC) and CCL5 (RANTES) [15, 16]. Adding chemokines to the tumor environment recruits TILs in vivo and decreases tumorigenicity [17]. Thus, inflammatory chemokines in the tumor microenvironment might improve the tumor infiltration by TCRV γ 9V δ 2⁺ T cells. Nevertheless, chemokines not only induce an interesting chemoattraction of immune cells but they also mediate angiogenesis, proliferation and metastasis of cancer cells [18]. A constitutive STAT3 activation in tumor cells limits the production of pro-inflammatory cytokines and CXCL10 and CCL5 chemokines [19]. Since STAT3 is a critical mediator of oncogenic signaling and angiogenesis in tumors, its therapeutic targeting could also benefit as an adjunct to the TCRV γ 9V δ 2⁺ T cell-based approaches [20]. Therefore, the type of chemokines detected in patients with cancer is a critical parameter for clinical trials.

Tumor angiogenesis determines lymphocyte infiltration, as the tumor vasculature is abnormal. Mice deficient for the Rgs5 gene, which contributes to this odd morphology, show normalized tumor vasculature, enhanced lymphoid infiltrates in tumors and improved survival [21]. Furthermore, anti-angiogenic drugs such as anti-VEGF antibodies or VEGFR inhibitors normalize the tumor vasculature in

patients and improve the delivery of antitumor drugs [22]. Therefore, such anti-angiogenic approaches could also benefit TCRV γ 9V δ 2⁺ T cell-based immunotherapies, and possibly represent an interesting therapeutic association to assess in the near future.

Immunosuppressive tumor stroma cells

Immature dendritic cells

Tumor blockade of DC maturation is certainly a major mechanism allowing tumor immune escape. Numerous studies of DC in mice and in human cancer tissues document the immature phenotype of tumor-infiltrating DC. This phenotype is characterized by a low expression of co-stimulatory molecules CD80/CD86 and a defective antigen presentation to $\alpha\beta$ T lymphocytes [23], though such features are also found in skin, peripheral blood and lymph node DCs from cancer patients [24, 25]. Instead of stimulating effective tumor antigen-specific $\alpha\beta$ T cell responses, these immature dendritic cells (iDC) rather induce T cell anergy and tumor tolerance [26]. iDCs potentiate TCRV γ 9V δ 2⁺ T cells response to phosphoantigen whereas, subsequently, phosphoantigen-stimulated TCRV γ 9V δ 2⁺ T cells promote efficient maturation of iDC and subsequent priming of $\alpha\beta$ T cell responses (by producing IFN γ and TNF α) [27, 28]. In addition, iDC are more potent co-stimulators of TCRV γ 9V δ 2⁺ T cell-derived cytokine production than mature DC, suggesting that tumor-induced iDC defects may not negatively impact TCRV γ 9V δ 2⁺ T cell antitumor responses [29]. Therefore, the TCRV γ 9V δ 2⁺ T cell-stimulating phosphoantigens might efficiently improve the functions of tumor vaccines by exerting an adjuvant bioactivity, although this type was not confirmed when tested in the context of a tuberculosis vaccine [30].

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC), a group of bone marrow-derived cells, are directly involved in the suppression of antitumor T cell responses in tumor-bearing mice (reviewed in [31]). These cells express Gr-1 and α M integrin CD11b, and accumulate in the spleen, the lymph node and the tumor tissues in mice. MDSC inhibit proliferation and IFN γ production of tumor antigen-specific CD8⁺ T lymphocytes. Similar expansion of human MDSC, defined by CD33⁺CD11b⁺ and the absence of other lymphoid and myeloid markers, has been observed in cancer patients [32, 33]. The exact mechanisms of tumor immune escape governed by MDSC, and whether they might modulate TCRV γ 9V δ 2⁺ T cell functions, remain unclear.

The possible mechanisms of MDSC-mediated suppression are listed below.

L-arginine-catabolizing arginase I (ARG1) is over-expressed in MDSC from tumor-bearing mice and cancer patients [34, 35], while L-arginine deprivation by MDSC impairs down-regulation of TCR-associated CD3 ζ chain, T cell activation and function. Arginase activity was increased in the CD11b⁺CD14[−] myeloid cells from 117 patients with renal cell carcinoma (RCC), and depletion of their MDSCs restored both T cell CD3 ζ expression and proliferative responses [34]. Thus, a similar MDSC-mediated deficit of L-arginine might also exhaust TCRV γ 9V δ 2⁺ T cells, contributing to the low efficacy of TCRV γ 9V δ 2⁺ T cell-based immunotherapies of advanced RCC [36, 37]. In addition, COX2-dependent production of prostaglandin E2 (PGE₂) by tumor cells also up-regulates ARG1 expression by MDSC [34, 35], suggesting that COX inhibitors also have some therapeutic potential for MDSC-targeting cancer immunotherapies.

Reactive oxygen species (ROS) including peroxynitrites (ONOO[−]) and hydrogen peroxide (H₂O₂) are also part of the MDSC's immunosuppressive arsenal [38]. Nitration of tyrosine residues from TCR/CD8 complexes by MDSC-derived peroxynitrite affects their conformational flexibility and subsequent interactions with peptide-CMH I complexes [39]. Likewise, the MDSC-mediated nitration of the TCRV γ 9V δ 2 might also reduce its binding to tumor-derived phosphoantigens. In such a case, the accumulation of MDSC in tumors might represent a means by which tumors attempt to escape to TCRV γ 9V δ 2⁺ T cell-based immunotherapies. Since, however, phosphodiesterase-5 (PDE5) inhibition with sildenafil, tadalafil, and vardenafil down-regulates ARG1 and NOS2 expression by MDSC and reduces their suppressive machinery in several mouse tumor models [31], PDE5 inhibitors are also susceptible to yield interesting anti-cancer associations with phosphoantigens.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent and uncommitted cells able to differentiate in many cell types, including osteoblasts, adipocytes, chondrocytes, tenocytes, or skeletal myocytes [40]. MSCs mostly reside in the bone marrow, but they are also distributed in the lymphoid, muscle and adipose tissues [40]. Their ability to regenerative functions in injured tissues are nevertheless associated with a potent immunosuppressive role [41]. MSCs escape immune recognition and inhibit $\alpha\beta$ T cell proliferation induced by mitogens or cognate peptides [42, 43]. They also inhibit the differentiation and maturation of DC [44] and NK cell proliferation [45]. The underlying immunosuppressive bioactivity of MSC involves cell

contact-dependent mechanisms and release of soluble factors such as PGE₂, IDO, NO, HGF as well as the TGF- β 1 and IL-10 cytokines [46].

MSCs are potent suppressors of TCRV γ 9V δ 2⁺ T cell functions including proliferation, cytokine (IFN γ and TNF α) production and tumor cell cytotoxicity. This results from the COX2-dependent production of PGE₂ by MSC, appearing as a dampening response to IFN γ and TNF α from TCRV γ 9V δ 2⁺ T cells [47, 48]. However, since PGE₂ immunosuppressive activity is mainly mediated by an AMPc-mediated PKA type I-dependent inhibitory signaling of the TCR activation cascade, this blockade can be reverted by indomethacin or by the PKA type I antagonist Rp-8-Br-cAMP [47, 49, 50]. The regulatory cross-talk between MSC and TCRV γ 9V δ 2⁺ T lymphocytes is therefore critical for $\gamma\delta$ T cell-based cancer immunotherapies.

MSCs are present in melanoma [51], glioma [52], carcinoma of the colon [53], ovaries [54], breast [55] and in the microenvironment of gastric carcinoma [56]. This presumably results from the potent MSC chemoattraction by the tumor-derived or stroma-derived MCP-1, SDF-1, PDGF, EGF and VEGF [57–59]. Stromal attraction of $\alpha\beta$ T cells—by the CXCL9, CXCL10 and CXCL11 chemokines—was found to be a tumoral strategy for a more efficient immunosuppression in mice [60]. Likewise, TCRV γ 9V δ 2⁺ T cells express the CXCR3 receptor for these chemokines [16], suggesting they can also be attracted in the tumor stroma by MSC to be silenced more efficiently with their inhibitory factors.

Regulatory T cells

About 1–3% of human CD4⁺ T lymphocytes are naturally occurring CD4⁺CD25⁺ regulatory T cells (Treg). These lymphocytes harbor cell surface markers of activated/memory T cells such as CD5^{hi}, CD45RB^{lo}, CTLA-4 (cytotoxic-T-lymphocytes-associated-protein 4) and GITR (glucocorticoid induced tumor necrosis factor receptor) (reviewed in [61]). They express the forkhead box P3 (Foxp3) transcription factor, a critical determinant of Treg development and function [62]. These cells, initially detected as inhibitors of self-reactive T cells [63], also inhibit immune responses against infections. Actually, Tregs are broad immunosuppressors which reduce the expansion and functions of cells of both innate and adaptive immunity [61].

Tregs inhibit phosphoantigen-induced TCRV γ 9V δ 2⁺ T cell proliferation in vitro and their depletion from peripheral blood mononuclear cells (PBMC) increases the *M. tuberculosis*-induced TCRV γ 9V δ 2⁺ T cells in tuberculin-positive individuals [64, 65]. Nevertheless, Tregs might or might not inhibit the TCRV γ 9V δ 2⁺ T cell cytokine and cytotoxic responses according to the strength of

their stimulating ligand, which comprised either phosphoantigen (BrHPP), anti-CD3 plus anti-CD28 mAbs or *M. tuberculosis*-derived ESAT-6 antigen in the various studies available as of now [65, 66].

Treg-mediated immunosuppression involves soluble factors and cellular mechanisms [67]. Cytokines such as IL-10, TGF- β [68] and IL-35 [69] mediate the Treg activity on T cell proliferation. Eventually, Treg cells can down-regulate antitumor responses through the perforin/granzyme B-dependent lysis of NK and CD8⁺ effector T cells [70]. Treg cells express the two adenosine-producing ectoenzymes CD39 and CD73 and adenosine inhibits T cell functions upon fixation to the A_{2A} receptor [71]. Finally, Tregs can also down-regulate antigen-presenting functions of DCs and promote their expression of IDO [72]. Treg suppression of TCRV γ 9V δ 2⁺ T cells most probably involves all these different mechanisms, but in this case might also involve a non-protein inhibitory factor that does not affect an Ag-specific CD8⁺ $\alpha\beta$ T cells proliferation [64]. Treg-mediated immunosuppression is clearly a major mechanism of tumor escape [73]. Treg cells were more abundant in blood, lymph nodes and tumors from 336 cancer patients than in healthy individuals. Their depletion restored the TCRV γ 9V δ 2⁺ T cell proliferation to phosphoantigen which was impaired in these cancer patients [64]. Therefore, dampening of Tregs functions might represent an important means to improve TCRV γ 9V δ 2⁺ T cell-mediated immunotherapy of cancer patients.

Since the first demonstration that Treg depletion improves antitumor immunity and tumor rejection in mice [74], many studies have targeted Tregs in cancer patients. The CD25-directed *Pseudomonas* immunotoxin LMB-2 and the denileukin diftotox hybrid protein (fusing IL-2 and diphtheria toxin) reduced Treg cells in cancer patients [75, 76]. A Treg-depleting vaccination (targeted against Foxp3) improved tumor immunity in mice with renal cell carcinomas [77] and the Treg-depleting cyclophosphamide boosts the efficacy of a DC vaccine in mice harboring melanoma or carcinoma [78]. In vivo, the IL-2-induced expansion of CD4⁺CD25⁺ Foxp3⁺ Treg cells can actually be countered by concurrent activation of TCRV γ 9V δ 2⁺ T lymphocytes by phosphoantigens. This not only down-regulates the expansion of IL-2-induced Tregs (presumably through competition for IL-2) but also improves Ag-specific T cell responses in a model of mycobacterial infection [79].

Immunosuppressive molecules

TGF- β

Transforming growth factor β (TGF- β) is a pivotal cytokine that is critically involved in peripheral tolerance and

resolution of inflammatory responses. It has the unique ability to regulate proliferation, differentiation and survival of T lymphocytes, NK, DCs and macrophages [80]. TGF- β is frequently detected in human cancer patients, where a high level of TGF- β is associated to a poor prognostic of the disease [81]. Various types of human tumor cells produce TGF- β , most probably to escape immune destruction through mechanisms which include, inter alia, inhibition of cytotoxicity by $\alpha\beta$ T and NK cells [82, 83]. TGF- β also inhibits proliferation and IFN γ production by *M. tuberculosis*-induced $\gamma\delta$ T cells [84], suggesting that TGF- β might also reduce these functions in cancer contexts.

We recently determined the ability of exogenous or tumor-derived TGF- β to interfere with TCRV γ 9V δ 2⁺ T cell antitumor functions and the underlying molecular mechanisms. We showed that TGF- β does not block their most upstream mechanisms of activation, in contrast with PGE₂. It rather inhibits their PAg/IL-2-induced proliferation and delays the progressive TCRV γ 9V δ 2⁺ T cell maturation into effectors without inducing Th17 or Treg-like $\gamma\delta$ cells. Hence, TGF- β reduces the TCRV γ 9V δ 2⁺ T cell cytotoxicity for target cancer cells, although increasing doses of the PAg stimulus or PAg plus the lymphoma-specific rituximab can counter TGF- β [85]. Therefore, PAg association with therapeutic mAbs has the therapeutic potential to reduce bioactivity of TGF- β [85], an objective currently sought by phase I/II clinical trials in glioblastoma and in systemic sclerosis with either antisense oligodeoxynucleotide inhibitors of TGF- β R-II kinase activity or with TGF- β -neutralizing antibodies.

Prostaglandin E₂

Prostaglandin E-2 (PGE₂) affects several facets of cancer growth such as cell proliferation, motility and apoptosis. In addition, PGE₂ inhibits DC, NK and $\alpha\beta$ T cell antitumor functions [86]. PGE₂ acts through its binding on seven-transmembrane domains containing receptors EP1, EP2, EP3 and EP4 coupled to different G proteins with different signaling pathways. EP-1 is coupled to Gq/p proteins, leading PGE₂ binding to increase the level of intracellular calcium. EP2 and EP4 are coupled to Gs proteins and induce intracellular cAMP accumulation by activating adenylate cyclase. Conversely, the Gi protein-coupled EP3 decreases intracellular cAMP concentrations [87]. We recently demonstrated that, by mediating an upstream blockade of TCRV γ 9V δ 2⁺ signals, PGE₂ is a critical regulator of all TCRV γ 9V δ 2⁺ T cell responses. Freshly isolated, resting TCRV γ 9V δ 2⁺ T cells and established TCRV γ 9V δ 2⁺ T cell lines strongly express the receptors EP2 and EP4 responsible for the PGE₂-mediated inhibition [47]. In CD4⁺ T cells, cAMP inhibits TCR signaling by PKA I (protein kinase A type I)-mediated phosphorylation

and activation of Csk (COOH-terminal Src kinase) which prevents Lck-mediated phosphorylation of the TCR-associated CD3 ITAMs (immunoreceptor tyrosine-based activatory motifs) [88]. In TCRV γ 9V δ 2⁺ T cells as well, PGE₂ irreversibly inhibits Lck-mediated phosphorylation of the TCR-associated CD3 ITAMs by the same PKA/cAMP-dependent mechanism [47].

Many human cancers produce high levels of PGE₂ due to up-regulated cyclooxygenase-2 (COX 2), a key enzyme in prostaglandin biosynthesis [89]. COX2 is detectable in more than 85% of colorectal adenocarcinomas whereas it is undetectable in normal colon mucosa [90]. High PGE₂ and COX2 levels are correlated with an adverse prognosis for many cancer patients. By its ability to prevent TCRV γ 9V δ 2⁺ T cell-mediated tumor cell lysis and secretion of pro-inflammatory cytokines, the tumor cell-derived PGE₂ could deeply impact the efficacy of TCRV γ 9V δ 2⁺ T cell-based immunotherapy. Moreover, the high serum levels of PGE₂ in most cancer patients [91] could participate in the impairment of their PAg-driven T cell proliferation [64, 92]. A correlation between PGE₂ levels or COX2 expression and TCRV γ 9V δ 2⁺ T cell responses in these patients nevertheless remain to be formally established. Selective COX2 inhibitors (COXibs) have proven efficacy for suppressing experimental tumorigenesis in mice and reducing incidence of colorectal adenomas in humans [93]. In addition, COX2ibs improves experimental $\alpha\beta$ T cell-based immunotherapies in mice [94]. Such approaches could also benefit to TCRV γ 9V δ 2⁺ T cell-based therapies. Of note, however, clinical trials of COXibs uncovered an increased risk for cardiovascular events [95], suggesting the need to develop more EP-selective drugs to counteract PGE₂ immunosuppression more safely.

Adenosine

Adenosine is formed from ATP, ADP, and AMP upon sequential enzymatic dephosphorylation in both intracellular and extracellular compartments. Extracellular adenosine concentrations are increased during tissue hypoxia, acute inflammation and in various cancers [96]. Adenosine binds to four different adenosine receptors A₁, A_{2A}, A_{2B} and A₃ that belong to the G-coupled seven-transmembrane domain superfamily. A_{2A} and A_{2B} signaling increase cAMP whereas conversely, A₁ and A₃ signaling decrease it. Tumor-derived adenosine inhibits antitumor $\alpha\beta$ T [97] and NK cells [98] through binding to their A_{2A} receptors. Experiments with A_{2A}^{KO} mice demonstrated that adenosine production is a significant immunoevasion pathway for malignant cells [97]. In addition, since the adenylate cyclase activator forskolin readily blocks TCRV γ 9V δ 2⁺ T cells responses, tumoral

adenosine is a most likely means to lower tumor cell destruction by TCRV γ 9V δ 2⁺ T cells as for the other cytolytic lymphocytes [47].

Indoleamine 2,3 dioxygenase

Indoleamine 2,3 dioxygenase (IDO), a cytosolic enzyme catabolizing tryptophan through the kynurenine pathway, is frequently detected in human tumor tissues and in tumor-draining lymph nodes [99, 100]. IDO expression is associated with a significant reduction of TILs and a poor clinical prognostic in ovarian [101] and colon carcinomas [102]. IDO-mediated starvation of extracellular L-tryptophan and accumulation of its L-kynurenine and picolinic acid catabolites inhibit proliferation of $\alpha\beta$ T lymphocytes [103] and affect NK cells functions, inter alia by down-regulation of the NKG2D and NKp46 receptors [104]. Whether IDO catabolites also inhibit anticancer TCRV γ 9V δ 2⁺ T cell functions has yet not been determined, but their metabolic needs matching those of $\alpha\beta$ T lymphocytes and NK cells render this possibility most likely.

Soluble NKG2D ligands

NKG2D binding to the MICA/B and ULBP1-4 ligands directly activates or co-stimulates $\gamma\delta$ T cell-mediated lysis of cancer cells [105, 106]. However, NKG2D ligands are often proteolytically shed from the surface of tumor cells to release these soluble molecules in the plasma from cancer patients [107]. High levels of soluble MICA/B and thus down-regulation of NKG2D have been associated with reduced tumoricidal functions of NK and tumour-specific CTL cells [107, 108]. So far, NKG2D expression defects on peripheral TCRV γ 9V δ 2⁺ T cells from cancer patients have not been reported. Nevertheless, soluble MIC molecules shed from pancreatic cancer cell lines diminish $\gamma\delta$ T cell cytotoxicity [109], suggesting that tumour-derived soluble forms of NKG2D ligands might be prone to dampen any TCRV γ 9V δ 2⁺ T cell-based cancer therapies.

Soluble HLA-G as ligands

The non-classical HLA class I molecule HLA-G has four membrane isoforms and three soluble isoforms. Various cancers (ovary, breast, colon, lung, melanoma) express high levels of HLA-G [110], an aberrant expression most likely induced upon oncogenic transformation by IL-10, IFN γ , kynurenine or HIF-1 α from the tumor niche, since normal tissues do not express it [111, 112]. Membrane-bound HLA-G directly inhibit the NK and CD8 T cell cytotoxicity as well as the proliferation and cytokine secretion from CD4 T cells [113]. This inhibition is

induced by the binding of membrane-bound HLA-G to the ILT2, ILT4, p49 and KIR2DL4 receptors on killer cells [114–116]. The binding of soluble HLA-G isoforms to CD8 and the inhibitory receptors ILT2, ILT4 and KIR2DL4 blocks cytotoxicity and induces Fas-mediated apoptosis of CD8⁺ and NK cells [117, 118]. Whether this is also true for cancer patients remains unknown.

IL-10

The tumor microenvironment recruits suppressive cells (tumor-associated macrophages, MSC) able to secrete immunosuppressive cytokines as TGF- β (see above) and/or IL-10. Thus, various tumors (lung, kidney, liver, colon) expressed high levels of IL-10 and this cytokine is also observed in the serum of cancer patients [119]. IL-10 inhibits innate and adaptive immunity cells. It blocks macrophage activation, DC-activation, -maturation and -functions, preventing the induction of specific response by $\alpha\beta$ T cells [120, 121]. IL-10 also inhibits IL-2-dependent proliferation and Th1 cytokine production by CD4⁺ T cells [119] and induces the decrease of MHC I or ICAM-1 molecule expressions, thereby altering recognition of tumor cells by CTL [122].

This cytokine inhibits TCRV γ 9V δ 2⁺ T cell in vitro proliferation induced by *M. tuberculosis* [123, 124]. However, other antigen stimuli such as the Daudi Burkitt's lymphoma cell line did not affect the TCRV γ 9V δ 2⁺ T cell proliferation in the presence of IL-10 [124]. IL-2 addition or the stimulus potency was able to counteract this inhibition. So, immunotherapies combining TCRV γ 9V δ 2⁺ T cells and mAbs could bypass immunosuppressive effect of both IL-10 and TGF- β cytokines.

FasL/TRAIL

Cytotoxic lymphocytes kill tumor cells by using, inter alia, the Fas/FasL or TRAILR/TRAIL pathways, cancer cells evolve by decreasing Fas or TRAILR expression as immunoevasion mechanism. Alternatively, Fas is altered, mutated, down-regulated or functionally deficient in many advanced cancers, which tumor cells also secrete the soluble receptor [125, 126]. Moreover, FasL or TRAIL molecules are over-expressed in many cancers, in relation to increased TIL apoptosis [127, 128]. The tumor microenvironment also triggers AICD by repeated TCR-stimulation [129, 130]. In this regard, the in vitro TCRV γ 9V δ 2⁺ T-cell amplification used for $\gamma\delta$ cell immunotherapy might offer a means to efficiently counter the tumor-induced AICD. In addition, FasL blockade could be combined with this cell immunotherapy since the Fas pathway is functional though dispensable in TCRV γ 9V δ 2⁺ cells.

Conclusions

Cancer grows thanks to a large spectrum of immuno-evasion strategies able to protect against $\gamma\delta$ T cells. Several other mechanisms, such as expression of PD-1 or CTLA-4 ligands, of galectins, and altered expression of other new TCRV γ 9V δ 2⁺ T cell-binding molecules, most likely might provide them with some immune privilege. Ongoing researches from our and other laboratories are currently addressing these questions with the aim of better success rates in future anticancer immunotherapies.

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