REVIEW

Human $\gamma\delta$ T-cell subsets and their involvement in tumor immunity

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$\gamma\delta$ T cells are a conserved population of innate lymphocytes with diverse structural and functional heterogeneity that participate in various immune responses during tumor progression. $\gamma\delta$ T cells perform potent immunosurveillance by exerting direct cytotoxicity, strong cytokine production and indirect antitumor immune responses. However, certain $\gamma\delta$ T-cell subsets also contribute to tumor progression by facilitating cancer-related inflammation and immunosuppression. Here, we review recent observations regarding the antitumor and protumor roles of major structural and functional subsets of human $\gamma\delta$ T cells, describing how these subsets are activated and polarized, and how these events relate to subsequent function in tumor immunity. These studies provide insights into the manipulation of $\gamma\delta$ T-cell function to facilitate more targeted approaches for tumor therapy.

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INTRODUCTION

$\gamma\delta$ T cells, which are innate-like T lymphocytes characterized by T-cell receptors (TCRs) composed of γ and δ chains, are widely distributed in the peripheral blood (PB) and mucosal tissues.¹ $\gamma\delta$ T cells rapidly recognize exogenous pathogens and endogenous stress-induced ligands in a major histocompatibility complex (MHC)-unrestricted manner and initiate adaptive immunity, acting as a first line of immune defense.² Activated $\gamma\delta$ T cells exhibit multiple effector functions, including cytotoxicity against infected or tumor cells, cytokine and chemokine production, antigen-presenting functions and regulatory abilities,³ thus allowing them to participate in an array of diseases, including infection, allergy, autoimmunity and cancer.^{4–6}

Human $\gamma\delta$ T cells contribute to the immune response against a subset of tumors of hematological and epithelial origin, and many clinical trials have been conducted to test the use of

$\gamma\delta$ T cells in adoptive cell therapy.⁷ However, human $\gamma\delta$ T cells have diverse physiological roles in tumor immunity, owing to their wide-ranging structural subsets, which are defined by their TCR repertoire and functional heterogeneity driven by differential environmental stimulation.^{8,9} Recent reports have described the diverse responses of human $\gamma\delta$ T cells to tumors.¹⁰ For example, $\gamma\delta$ T cells exert cytotoxicity toward tumor cells via the NKG2D pathway,¹¹ however, they also develop a regulatory profile by expressing interleukin-10 (IL-10) and tumor growth factor (TGF)- β , thereby exerting suppressive effects on antitumor responses.¹² Moreover, our previous studies have indicated that human PB V δ 1 T cells demonstrate favorable cytotoxicity against colon cancer,¹³ whereas $\gamma\delta$ T17 cells with V δ 1 TCR usage in colon cancer tissue promote tumor progression.¹⁴

Therefore, understanding $\gamma\delta$ T-cell subset-specific responses during tumor immunity is vital to rationally exploit the

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antitumor activity of $\gamma\delta$ T cells while avoiding their tumor-promoting effects during tumor therapy. In this review, we summarize research progress regarding the major structural and functional subsets of human $\gamma\delta$ T cells and their effects on tumor immunity, and we describe the clinical implications for tumor therapy involving the manipulation of $\gamma\delta$ T-cell function.

STRUCTURAL SUBSETS AND $\gamma\delta$ T-CELL ACTIVATION

Generally, human $\gamma\delta$ T cells are divided into two major structural subsets according to their TCR δ chain usage: V δ 1 and V δ 2 T cells.¹⁵ In terms of TCR γ chain usage, V δ 1 T cells are predominantly associated with the V γ I gene family (V γ 2/3/4/5/8), whereas the majority of V δ 2 T cells coexpress V γ II (V γ 9).¹⁶ $\gamma\delta$ T subsets exhibit distinct developmental properties, tissue localization and activation modes.^{1,17,18}

V γ 9V δ 2 $\gamma\delta$ T CELLS

$\gamma\delta$ T-cell development primarily occurs in the fetal thymus, and subsets arise through rearrangements at distinct phases of thymic ontogeny.¹⁹ V δ 2 subsets are generated in the thymus at 8.5–15 weeks in human embryos, with gene rearrangements of V δ 2 to D δ 3 and of V γ 1.8 or V γ 9 to J γ 1.¹⁹ Human V δ 2 T cells, which are almost exclusively paired with the V γ 9 chain (also termed V γ 9V δ 2 $\gamma\delta$ T cells), are predominant in the PB (>70%),¹⁵ and are uniquely activated by phosphoantigens produced by microbes and transformed cells. Exposure to (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), an intermediate metabolite of microbial isoprenoid biosynthesis²⁰ and Isopentenyl pyrophosphate (IPP), which is generated by transformed mammalian cells via the mevalonate pathway, leads to TCR-dependent activation of V γ 9V δ 2 T cells,²¹ thus enabling them to rapidly respond to exogenous infection or endogenous transformed cells. Moreover, aminobisphosphonates such as zoledronic acid combined with low-dose IL-2 selectively activate and expand V γ 9V δ 2 T cells *in vitro*.²² Phosphoantigens interact with specific proteins rather than being directly recognized by the TCR.²³ F1-ATPase expressed on tumor cells has been defined as an antigen-recognition molecule for phosphoantigen-mediated stimulation of human V γ 9V δ 2 T cells.²⁴ Butyrophilin3A1 is another essential phosphorylated antigen-presenting modality of V γ 9V δ 2 T-cell activation.^{25–27} In addition to phosphoantigens, human MutS homolog 2, a DNA repair-related protein ectopically expressed on tumor cells, is recognized by V γ 9V δ 2 T cells via the TCR.²⁸

Toll-like receptors (TLRs) and natural killer receptors (NKR) have been reported to co-stimulate human V γ 9V δ 2 T cells in combination with TCR stimulation.^{29,30} Pathogen-associated molecular patterns derived from microbes trigger V γ 9V δ 2 T-cell activation via TLRs and promote cytokine and chemokine production.²⁹ Moreover, human V γ 9V δ 2 T cells also recognize stress-induced MHC class I chain-related antigens A and B (MICA/B) as well as MIC-A-related UL16-binding proteins (ULBPs) upregulated by transformed or infected cells via NKG2D.¹¹ Another NKR involved in V γ 9V δ 2 T-cell activation, DNAM-1, binds to its ligand, nectin-like-5, which is expressed on tumor cells, and consequently exerts

cytotoxic effects.³¹ V γ 9V δ 2 T cells also respond to superantigens such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST)-1.^{32,33} The above evidence has demonstrated that V γ 9V δ 2 T cells respond to a variety of ligands, although these represent only a few defined antigens; these responses suggest implications for the clinical management of these cells.

V δ 1 $\gamma\delta$ T CELLS

V δ 1 TCR gene rearrangement occurs 4–6 months after birth and involves the joining of V δ 1 to D δ 1 or D δ 2 and the joining of upstream V γ gene segments, including V γ 2, 3, 5 and 8, to J γ 2.¹⁹ Unlike V γ 9V δ 2 T cells, human V δ 1 T cells primarily reside in the gut epithelia, dermis, spleen and liver, and are involved in maintaining epithelial tissue integrity.¹ V δ 1 T cells constitute less than 30% of $\gamma\delta$ T cells in PB and contain diverse paired V γ chains.^{15,16} During HIV infection, V δ 1 T-cell numbers are increased, and the normal ratio of V δ 2/V δ 1 T cells is inverted, thus suggesting the potential involvement of V δ 1 T cells in antiviral immunity.³⁴ Ligand recognition by V δ 1 T cells remains largely uncharacterized, although CD1 family proteins are recognized by V δ 1 T cells. Both PB and tissue V δ 1 T cells recognize CD1c^{35–37} and the lipid-presenting MHC-like molecule CD1d via the TCR.³⁸ Two recent studies have explored the structural basis of the recognition of lipid antigens by the V δ 1 TCR via CD1d-presenting molecules.^{39,40} In addition to the CD1 family, human intestinal epithelial V δ 1 T cells respond to stress-induced MICA/B through the synergistic actions of TCR and NKG2D.^{41,42} Specifically, in a manner analogous to V γ 9V δ 2 T cells, V δ 1 T cells respond to tumor cells by overexpressing MICA/B and ULBPs via NKG2D.^{43,44} Moreover, V δ 1 T cells are activated by the superantigen SE but respond exclusively to SEB rather than SEA.⁴⁵ A unique feature of V δ 1 T-cell activation is the recognition of B7-H6, a B7 family member exclusively expressed on tumor cells, by NKp30, thereby exerting anti-tumor effects.^{46,47}

NON-V δ 1 AND NON-V γ 9V δ 2 $\gamma\delta$ T CELLS

Human V δ 3 T cells compose the majority of non-V δ 1 and non-V γ 9V δ 2 $\gamma\delta$ T cells and are found in healthy PB, the liver⁴⁸ and in patients with cytomegalovirus (CMV) infection,⁴⁹ HIV infection⁵⁰ and B-cell leukemia.⁵¹ V δ 3 T cells, paired with V γ 2 or V γ 3,⁵⁰ respond to CD1d and express the degranulation marker CD107a.⁵² A V γ 4V δ 5+ T-cell clone has been reported to recognize stressed human cells via TCR binding to endothelial protein C receptor.⁵³ Furthermore, V δ 4, V δ 6, V δ 7 and V δ 8 T cells have been detected in the PB of lymphoma patients;⁵⁴ however, further studies are required to evaluate γ chain pairings and how these subsets are activated. Studies examining the activation of $\gamma\delta$ T-cell subsets are highlighted in Table 1.

FUNCTIONAL SUBSETS AND $\gamma\delta$ T-CELL POLARIZATION

$\gamma\delta$ T cells share pleiotropic functions with conventional $\alpha\beta$ T cells.⁵⁵ Each functional subset is induced through the

Table 1 Structural subsets of human $\gamma\delta$ T cells

Structural subset	Paired V_γ gene usage	Distribution	Activation stimulus and/or $\gamma\delta$ TCR ligands	References
V δ 1	V γ 2/3/4/5/8/9	PB, skin, gut, spleen, liver	MICA/B; ULBPs; B7-H6; CD1c; CD1d; SEB	35,39,44–46
V δ 2	V γ 9	PB	Phosphoantigens; F1-ATPase; BTN3A1; hMSH2; MICA/B; ULBPs; SEs; TSST-1; Nectin-like-5;	20,24,27–28,31–33
V δ 3	V γ 2/3	PB, liver	CD1d	50,52
V δ 5	V γ 4	PB	EPCR	53

Abbreviations: BTN3A1, butyrophilin3A1; EPCR, endothelial protein C receptor; hMSH2, human MutS homolog 2; MHC, major histocompatibility complex; MICA/B, MHC class I chain-related antigens A and B; PB, peripheral blood; ULBP, UL16-binding protein; SE, staphylococcal superantigens; TSST-1, toxic shock syndrome toxin-1.

stimulation of resting $\gamma\delta$ T cells by different polarization factors *in vitro*.⁵⁶

IFN- γ -PRODUCING $\gamma\delta$ T CELLS

Human circulating $\gamma\delta$ T cells are driven to produce interferon (IFN)- γ in the presence of IPP by IL-12 and anti-IL-4 antibodies, whereas these cells are polarized and become IL-4-producing cells when exposed to IPP plus IL-4 and anti-IL-12 antibodies,⁵⁷ which mediate anti-infection responses. Moreover, activation of an IFN- γ -producing response in the absence of IL-4 detection is promoted by nonpeptide antigens plus IL-21.⁵⁸ Similarly, IL-2 and IL-21 drive $\gamma\delta$ T cells toward an IFN- γ -producing phenotype characterized by increased CD56 expression and enhanced cytolytic responses.^{59,60} IL-2 and IL-15 signals drive human $\gamma\delta$ T-cell differentiation toward cytotoxic IFN- γ -producing subsets in the absence of TCR activation.⁶¹

ANTIGEN-PRESENTING $\gamma\delta$ T CELLS

$\gamma\delta$ T cells also display functional plasticity in terms of indirect anti-infection or antitumor responses.^{62,63} Bovine $\gamma\delta$ T cells present antigens to CD4+ $\alpha\beta$ T cells.⁶⁴ Microbial infections induce professional antigen-presenting cell (APC) functions of human tonsillar $\gamma\delta$ T cells characterized by the expression of co-stimulatory molecules such as MHC-II, CD80, CD86 and CD40, thereby initiating adaptive immune responses by CD4+ and CD8+ $\alpha\beta$ T cells.⁶⁵ Furthermore, $\gamma\delta$ T-APCs process soluble protein for cross-presentation on MHC-I and induce CD8+ $\alpha\beta$ T-effector cell responses more efficiently than monocyte-derived dendritic cells (DCs).⁶⁶

FOLLICULAR B HELPER $\gamma\delta$ T CELLS

Follicular T helper (T_{FH}) cells have critical roles in adaptive immunity via interactions with B cells.⁶⁷ Vermijlen D *et al*.⁵⁶ have reported IL-21-induced expression of the follicular B-cell-attracting chemokine CXCL13/BCA-1 on $\gamma\delta$ T cells, thus resulting in a T_{FH} -associated phenotype. The transcriptional suppressor Bcl-6 is an indispensable regulator of T_{FH} lineage commitment.⁶⁷ $\gamma\delta T_{FH}$ cells polarized by HMB-PP and IL-21 exhibit T_{FH} -like activity accompanied by the expression of the transcriptional repressors Bcl-6, ICOS, CD40L, CXCR5, IL-21R, CD244, CXCL10 and CXCL13, which, in maturing B cells, facilitate the production of high-affinity antibodies against foreign antigens.^{68,69}

REGULATORY $\gamma\delta$ T CELLS

$\gamma\delta$ T cells also exert immunosuppressive and regulatory activities during immune responses. Casetti *et al*.⁷⁰ have reported the induction of Foxp3+ regulatory $\gamma\delta$ T ($\gamma\delta$ Treg) cells by TGF- β 1 and IL-15, accompanied by antigen stimulation, which inhibits the proliferation of anti-CD3 and anti-CD28 antibody-stimulated PBMCs. Indeed, *in vitro*-expanded V δ 1 T cells stimulated by an anti-human TCR V δ 1 antibody with TGF- β 1 predominantly express Foxp3, CD25, glucocorticoid-induced TNFR family-related protein and CTLA4, all of which suppress CD4+ T cell proliferation.⁷¹ Tumor-infiltrating $\gamma\delta$ Treg cells are induced by IP-10 secreted by breast cancer cells, thereby suppressing T-cell responses and DC maturation.⁷² These regulatory $\gamma\delta$ T cells lack the expression of Foxp3, GIRT and CD25, and their suppressive activity does not occur via TGF- β or IL-10.⁷³ Recently, we have identified a novel $\gamma\delta$ Treg subset exhibiting CD39 expression that accounts for 60% of $\gamma\delta$ T17 cells and is polarized by TGF- β , thus resulting in stronger immunosuppression than CD4+ Treg cells in the context of human colorectal cancer (unpublished data). These CD39+ $\gamma\delta$ Treg cells suppress the activity of human CD3+ T cells in an adenosine-dependent manner (unpublished data).

IL-17-PRODUCING $\gamma\delta$ T CELLS

$\gamma\delta$ T17 cells broadly participate in inflammatory responses, having pathogenic roles during infection and autoimmune diseases.⁷⁴ Differentiation into $\gamma\delta$ 17 T cells requires high levels of RAR-related orphan receptor C (RORC) and aryl hydrocarbon receptor (AHR) expression but low levels of T-bet expression, which is efficiently induced by coordinated stimulation by phosphoantigens and cytokines, including IL-1 β , TGF- β , IL-6 and IL-23.⁷⁵ Fresh human cord blood $\gamma\delta$ T cells cultured with IL-7 plus TCR agonists for 1 week and stimulated by PMA and ionomycin for 6 h were polarized into IL-17 producers.⁷⁶ IL-6, IL-1 β and TGF- β are required to generate $\gamma\delta$ T17 cells in neonates.⁷⁷ In addition, IL-23 is highly important for $\gamma\delta$ T17 cell maturation and growth.⁷⁸ In a previous study, we have identified that $\gamma\delta$ T17 cells polarized in human colorectal cancer tissue under stimulation by IL-23 derived from inflammatory DCs.¹⁴ Table 2 summarizes studies investigating the polarization of $\gamma\delta$ T-cell subsets with distinct functions.

Table 2 Functional subsets of human $\gamma\delta$ T cells

Functional subsets	Polarization	References
IFN- γ -producing $\gamma\delta$ T	IPP+IL-12+IL-4 antibody; IL-2+IL-21; nonpeptide antigens+IL-21; IL-2+IL-15	57–61
IL-4-producing $\gamma\delta$ T	IPP+IL-4+IL-12 antibody	57
$\gamma\delta$ T-APC	Microbial product	6
$\gamma\delta$ T _{FH}	IL-21; HMB-PP+IL-21	56,68
$\gamma\delta$ Treg	TGF- β +IL-15; V δ 1 TCR antibody+ TGF- β 1; IP-10	70–73
$\gamma\delta$ T17	IL-7+TCR agonists; IL-23; phosphoantigens+IL-1 β +TGF- β +IL-6+IL-23; IL-6+IL-1 β +TGF- β	14,75–77

Abbreviations: APC, antigen-presenting cell; $\gamma\delta$ Treg, regulatory $\gamma\delta$ T; HMB-PP, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; IFN, interferon; IL, interleukin; IPP, Isopentenyl pyrophosphate; TCR, T-cell receptor; TGF, tumor growth factor.

Figure 1 Polarization and responses of human $\gamma\delta$ T-cell subsets to tumors.

THE ROLE OF $\gamma\delta$ T-CELL SUBSETS IN TUMOR IMMUNITY

Differentially polarized $\gamma\delta$ T-cell subsets exhibit functionally diverse responses to tumors, thus potentially leading to antitumor or protumor responses (Figure 1).

ANTITUMOR EFFECTS

The first report of tumor surveillance by $\gamma\delta$ T cells described a potential association between the increased frequency of $\gamma\delta$ T cells and improved disease-free survival of leukemia patients who received $\alpha\beta$ T-cell-depleted bone marrow transplants.⁷⁹ Recently, intratumoral $\gamma\delta$ T cells have been demonstrated to be the most significant predictors of favorable survival across various cancer types.⁸⁰ $\gamma\delta$ T cells display cytotoxicity against hematopoietic and solid tumors in an MHC-independent manner.⁸ Although their activation mechanisms differ, both V δ 2 and V δ 1 subsets exert potent antitumor effects.⁸ One common $\gamma\delta$ T-cell-mediated killing pattern involves tumor cell recognition via receptor–ligand interactions. TCR is strongly implicated in controlling V γ 9V δ 2 T-cell cytotoxicity via the recognition of phosphoantigens that are overexpressed in tumor cells and mediate tumor cell lysis.⁸¹ NKG2D binds to MICA/B and ULBPs and induces V γ 9V δ 2 T-cell cytotoxicity against hemopoietic and epithelial tumors.^{11,30,82–84} V γ 9V δ 2 T cells are induced to produce IFN- γ and kill hepatocellular carcinoma cells via the interaction of DNAM-1 and nectin-like-5.³¹ $\gamma\delta$ T cells also exhibit strong cytotoxicity against myeloma cells via NKp44.⁸⁵ Furthermore,

CD56+ $\gamma\delta$ T cells are capable of killing squamous cell carcinoma of the head and neck, a process that is likely to be mediated by the enhanced expression of granzyme B and upregulated degranulation.⁸⁶

Similarly to NK cells, $\gamma\delta$ T cells induce antibody-dependent cell-mediated cytotoxicity (ADCC) effects, thus resulting in the lysis of tumor cells. According to Tokuyama H *et al.*,⁸⁷ CD16+ V γ 9V δ 2 T cells recognize monoclonal antibody-coated lymphoma, chronic lymphocytic leukemia (CLL) and breast cancer cells via CD16 and exert ADCC-dependent cytotoxicity. $\gamma\delta$ T cells mediate ADCC against B-lineage acute lymphoblastic leukemia via CD19 antibodies.⁸⁸ In several other studies, $\gamma\delta$ T cells have also been shown to mediate ADCC effects against tumor cells via CD16 in the presence of therapeutic antitumor monoclonal antibodies.^{89–91}

Moreover, $\gamma\delta$ T cells have antitumor roles by modulating other effector cells. For instance, V γ 9V δ 2 T cells process endogenous antigens along the MHC-I peptide presentation pathway, which may promote antitumor adaptive immunity via the cross-presentation of tumor antigens.⁶⁵ V γ 9V δ 2 T cells activated by HMB-PP promote Th1 responses by inducing DC maturation and IL-12 secretion, which may facilitate antitumor immunity.⁹² IPP-expanded V γ 9V δ 2 T cells induce NK cells to recognize and kill tumors that are usually resistant to NK cytotoxicity by increasing NKG2D expression on their surface through CD137L co-stimulation.⁹³ Phosphoantigen-activated APC-like V γ 9V δ 2 T cells present glycolipid antigens to

invariant NKT cells in a CD1d-restricted and α -GalCer-dependent manner, and subsequently initiate antitumor responses.⁹⁴ Together, these results suggest that V γ 9V δ 2 T cells exert antitumor effects primarily through direct killing, ADCC-dependent cytotoxicity and by regulating the functions of other innate and adaptive immune cells.

The dramatic expansion of V δ 1 T cells, which usually compose a minor proportion of PB $\gamma\delta$ T cells, has been observed in solid organ transplant recipients who had developed CMV infection,^{95,96} and the long-term expansion of effector V δ 1 T cells is a specific blood signature of CMV infection.⁹⁷ Anti-CMV-reactive V δ 1 T cells recognize intestinal tumor epithelial cells. After recognition, V δ 1 T cells release IFN- γ and tumor necrosis factor- α (TNF- α) and exert FasL-, TNF- α -independent and perforin-dependent cytotoxicity against target cells.⁹⁸ CMV-induced V δ 1 T cells demonstrate better antitumor potential and are associated with reduced cancer risk in kidney transplant recipients.⁹⁹ Expanded V δ 1 T cells expressing CD8 $\alpha\alpha$ after CMV reactivation after allogeneic stem cell transplantation recognize both CMV-infected cells and primary leukemic blasts.¹⁰⁰ In contrast, *ex vivo*-expanded V δ 1 T cells mediate the killing of glioblastoma cells in a CMV-independent manner.¹⁰¹ Furthermore, CMV infection also decreases tumor immunogenicity by downregulating the expression of NKG2D ligands and ULBPs.^{102,103} Together, these results indicate that CMV infection is closely associated with the antitumor immunity of V δ 1 T cells, although the mechanism underlying the recognition of CMV-infected cells and tumor cells by V δ 1 T cells requires further study.

In addition to CMV-associated antitumor activity, both circulating and tumor-infiltrating V δ 1 T cells respond to malignancies of hematological and epithelial origin. Circulating V δ 1 T cells contribute to the antitumor response against low-grade non-Hodgkin lymphoma (NHL) by recognizing ULBPs on lymphoma cells.⁴⁴ Moreover, V δ 1 T cells, but not V γ 9V δ 2 T cells, have been detected in ULBP-positive lymph nodes in NHL patients.⁴⁴ In our previous study, we have found that *ex vivo*-expanded human PB V δ 1 T cells demonstrate more potent killing of colon cancer cells than V γ 9V δ 2 T cells via cytolytic receptor–ligand interactions.¹³ Moreover, human V δ 1 T cells have been reported to inhibit tumor metastases independently of primary tumor control in a xenograft model of colon cancer.¹⁰⁴ Tumor-infiltrating V δ 1 T cells isolated from colorectal cancer exert cytotoxicity against autologous and allogeneic cancer cells via the recognition of cell surface antigens shared by epithelial tumors.¹⁰⁵ With proper induction, *In vitro*-re-activated tumor-infiltrating V δ 1 T cells isolated from melanoma produce TNF- α and IFN- γ , and act in a cytolytic manner against tumor cells.^{106,107} *Ex vivo*-expanded V δ 1 T cells isolated from various solid tumors demonstrate stronger cytotoxicity against tumor cell lines and/or freshly isolated tumor cells compared with V γ 9V δ 2 T cells.^{105,108–112} Notably, in a previous study, the majority of V δ 1 T-cell lines exerted robust cytotoxic responses against the melanoma cell line A375, whereas only two of eight V δ 2 T-cell lines demonstrated clear cytotoxic activity against A375, which was

enhanced by pretreating target cells with zoledronate.¹⁰⁷ Thus, although both structural subsets of $\gamma\delta$ T cells exert antitumor effects, V δ 1 T cells are potentially better killers than V γ 9V δ 2 T cells, at least in the context of certain tumors.

PROTUMOR EFFECTS

Although $\gamma\delta$ T cells demonstrate potent antitumor capacity, paradoxically they also exert protumor effects by promoting noncytotoxic inflammation and regulatory functions that subvert cytotoxic antitumor immunity. Intratumoral $\gamma\delta$ T cell numbers are positively associated with advanced tumor stages and are inversely correlated with breast cancer prognosis.¹¹³ $\gamma\delta$ T cells are essential producers of IL-17, both in mice and humans.^{75,114} Furthermore, IL-17 mediates inflammatory responses in tumor immunity. In our previous review, we have described how IL-17 promotes colorectal cancer progression.¹¹⁵ According to recent studies, $\gamma\delta$ T17 cells exert tumor-promoting effects in mice by facilitating angiogenesis.^{114,116} $\gamma\delta$ T17 cells also promote breast cancer metastasis because mice treated with $\gamma\delta$ T-cell-depleting agents or anti- $\gamma\delta$ TCR antibodies are profoundly protected against pulmonary and lymph node metastases.¹¹⁷ However, there have been few studies investigating the role of human $\gamma\delta$ T17 cells in tumor immunity. In our previous study, we have found that tumor-infiltrating $\gamma\delta$ T17 cells induced by tumor-elicited inflammation promote tumor progression via the secretion of IL-17, IL-8, tumor necrosis factor- α (TNF- α) and granulocyte-macrophage colony-stimulating factor (GM-CSF), thereby forming an immunosuppressive microenvironment in human colorectal cancer.¹⁴ Furthermore, $\gamma\delta$ T17 cells are the predominant producers of IL-17 in lung cancer (unpublished data), thus indicating their crucial role in IL-17-related inflammatory responses in tumor immunity. In a murine ovarian cancer model, $\gamma\delta$ T17 cells have been found to accumulate during later stages of tumor progression.¹¹⁸ We have also demonstrated a positive correlation between $\gamma\delta$ T17 cell numbers and advancing tumor stages of human colorectal cancer.¹⁴

$\gamma\delta$ T cells possess potential regulatory roles in the control of tumor immune responses. For example, according to Peng *et al.*,⁷³ tumor-infiltrating $\gamma\delta$ T cells in breast cancer contribute to the formation of an immunosuppressive microenvironment by suppressing naive and effector T cells and impairing DC maturation and function. In addition, $\gamma\delta$ Treg cells derived from breast cancer induce the immunosenescence of naive and effector T cells and DCs, and this immunosuppressive activity is further amplified by the senescent cells themselves.¹¹⁹ Moreover, our group has identified a novel $\gamma\delta$ Treg subset in human colorectal cancer that promotes an immunosuppressive microenvironment via a metabolism-related mechanism (unpublished data). Thus, certain $\gamma\delta$ T cell subsets behave as immunosuppressive cells and promote tumor progression in specific cancers. However, more studies focusing on the polarization mechanisms of protumor $\gamma\delta$ T cells in human tumor microenvironments (TMEs) are needed.

CLINICAL IMPLICATIONS

Given their potent MHC-unrestricted antitumor effector activities, $\gamma\delta$ T cells are attractive candidates for antitumor immunotherapies. The cytotoxic features of the V δ 1 and V δ 2 subsets have been investigated.⁸ Preclinical and clinical studies have paved the way for V γ 9V δ 2 T-cell-mediated immunotherapy, given the high-frequency and broad antitumor properties of this cell type.¹²⁰ Clinical-scale expansion of V γ 9V δ 2 T cells via direct stimulation by phosphoantigens or the induction of agonist accumulation with aminobisphosphonates makes V γ 9V δ 2 T-cell-based cancer immunotherapy feasible.¹²⁰ Phase I and II clinical trials have been conducted in patients with various tumor types, and objective tumor responses have been observed.⁷ Given the accumulating evidence supporting the cytotoxic functions of V δ 1 subsets in basic research,^{13,121,122} V δ 1 T cells may be a potent tool for clinical manipulation in cancer immunotherapy, and efforts have been put forth to explore strategies for clinical-grade expansion. Intriguingly, IL-4 promotes the proliferation of V δ 1 T cells and simultaneously inhibits V δ 2 T-cell growth,¹²³ thus providing a novel basis to develop preferential expansion approaches for V δ 1 T cells. Recently, Almeida *et al.*¹²⁴ have reported a robust two-step protocol for the selective expansion of V δ 1 T cells up to 2000-fold, and cellular products demonstrated strong cytotoxicity *in vitro* and therapeutic potential in xenograft models of CLL. Clinical trials are necessary to ascertain the safety and efficacy of V δ 1 T cells to move forward with autologous or allogeneic cell therapies for both hematological and solid tumors.

Immunosuppressive functions of $\gamma\delta$ T cells infiltrating breast cancer and colorectal cancer TMEs have been described.^{14,73} The emerging evidence supporting protumor roles for specific $\gamma\delta$ T-cell subsets potentially poses an obstacle to the development of future therapies.¹²⁵ Although knowledge of $\gamma\delta$ T-cell function in the TME has gradually increased, it remains a challenge to determine whether the inflammatory and regulatory features of $\gamma\delta$ T cells in the tumor-infiltrating lymphocytes are intrinsic or induced by inflammatory factors in the TME. To achieve successful therapeutic effects, it may be better to identify immunosuppressive functional subsets and eliminate them from a population of adoptive $\gamma\delta$ T cells before transfer or to combine $\gamma\delta$ T-cell-based adoptive immunotherapy with a strategy targeting the TME to prevent potential polarization into tumor-promoting subsets.

CONCLUDING REMARKS

There are no clear boundaries between the structural and functional subsets of $\gamma\delta$ T cells, and it is possible to polarize V δ 2 T cells into nearly all functional subsets. However, efforts should be made to further distinguish between V δ 1 and V δ 2 subsets, which may differ substantially in terms of their localization and demonstrate context-dependent plasticity and function. To date, no one-to-one correspondence between a specific TCR structure and a specific effector $\gamma\delta$ T-cell type has been reported. A myriad of evidence indicates either antitumor effects or tumor-promoting activities for $\gamma\delta$ T cells in tumor

immunity. The dual role of $\gamma\delta$ T cells is closely associated with their complex surrounding microenvironment, which influences $\gamma\delta$ T-cell polarization. Our group has identified the ability of *ex vivo*-expanded V δ 1 T cells to exert favorable killing activity against colon cancer, whereas $\gamma\delta$ T17 cells in colon cancer tissue, the majority of which demonstrate V δ 1 TCR usage, promote the formation of an immunosuppressive TME and thus exert a tumor-promoting role. Therefore, deciphering the mechanisms underlying the development, tissue tropism, ligands and immune responses of $\gamma\delta$ T-cell subsets should elucidate their effects in tumor immunity, thus providing sufficient evidence for the application of $\gamma\delta$ T-cell subsets for antitumor adoptive immunotherapy or for targeting certain inflammatory or regulatory $\gamma\delta$ T-cell subsets for tumor therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

DW and PW contributed to the literature collection and manuscript writing. Fuming Qiu contributed to manuscript polishing. JH and QW participated in the design and review of the manuscript.

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