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REVIEW

Human γδT-cell subsets and their involvement in tumor immunity

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γδT cells are a conserved population of innate lymphocytes with diverse structural and functional heterogeneity that participate in various immune responses during tumor progression. γδT cells perform potent immunosurveillance by exerting direct cytotoxicity, strong cytokine production and indirect antitumor immune responses. However, certain γδT-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 γδT 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 γδT-cell function to facilitate more targeted approaches for tumor therapy.
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INTRODUCTION

γδT 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] γδT 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 γδT 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 γδT 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 γδT cells in adoptive cell therapy.[7] However, human γδT 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 γδT cells to tumors.[10] For example, γδT 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 Vδ1 T cells demonstrate favorable cytotoxicity against colon cancer,[13] whereas γδT17 cells with Vδ1 TCR usage in colon cancer tissue promote tumor progression.[14]

Therefore, understanding γδT-cell subset-specific responses during tumor immunity is vital to rationally exploit the

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antitumor activity of γδT 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 γδT cells and their effects on tumor immunity, and we describe the clinical implications for tumor therapy involving the manipulation of γδT-cell function.

STRUCTURAL SUBSETS AND γδT-CELL ACTIVATION

Generally, human γδT cells are divided into two major structural subsets according to their TCR δ chain usage: Vδ1 and Vδ2 T cells.[15] In terms of TCR γ chain usage, Vδ1 T cells are predominantly associated with the VγI gene family (Vγ2/3/4/5/8), whereas the majority of Vδ2 T cells coexpress VγII (Vγ9).[16] γδT subsets exhibit distinct developmental properties, tissue localization and activation modes.[1,17,18]

Vγ9Vδ2 γδT CELLS

γδT-cell development primarily occurs in the fetal thymus, and subsets arise through rearrangements at distinct phases of thymic ontogeny.[19] Vδ2 subsets are generated in the thymi at 8.5–15 weeks in human embryos, with gene rearrangements of Vδ2 to Dδ3 and of Vγ1.8 or Vγ9 to Jγ1.[19] Human Vδ2 T cells, which are almost exclusively paired with the Vγ9 chain (also termed Vγ9Vδ2 γδT 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 biosynthesis[20] and Isopentenyl pyrophosphate (IPP), which is generated by transformed mammalian cells via the the mevalonate pathway, leads to TCR-dependent activation of Vγ9Vδ2 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 Vγ9Vδ2 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 Vγ9Vδ2 T cells.[24] Butyrophilin3A1 is another essential phosphorylated antigen-presenting modality of Vγ9Vδ2 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 Vγ9Vδ2 T cells via the TCR.[28]

Toll-like receptors (TLRs) and natural killer receptors (NKRs) have been reported to co-stimulate human Vγ9Vδ2 T cells in combination with TCR stimulation.[29,30] Pathogen-associated molecular patterns derived from microbes trigger Vγ9Vδ2 T-cell activation via TLRs and promote cytokine and chemokine production.[29] Moreover, human Vγ9Vδ2 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 Vγ9Vδ2 T-cell activation, DNAM-1, binds to its ligand, nectin-like-5, which is expressed on tumor cells, and consequently exerts cytotoxic effects.[31] Vγ9Vδ2 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 Vγ9Vδ2 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.

Vδ1 γδT CELLS

Vδ1 TCR gene rearrangement occurs 4–6 months after birth and involves the joining of Vδ1 to Dδ1 or Dδ2 and the joining of upstream Vγ gene segments, including Vγ2, 3, 5 and 8, to Jγ2.[19] Unlike Vγ9Vδ2 T cells, human Vδ1 T cells primarily reside in the gut epithelia, dermis, spleen and liver, and are involved in maintaining epithelial tissue integrity.[1] Vδ1 T cells constitute less than 30% of γδT cells in PB and contain diverse paired Vγ chains.[15,16] During HIV infection, Vδ1 T-cell numbers are increased, and the normal ratio of Vδ2/Vδ1 T cells is inverted, thus suggesting the potential involvement of Vδ1 T cells in antiviral immunity.[34] Ligand recognition by Vδ1 T cells remains largely uncharacterized, although CD1 family proteins are recognized by Vδ1 T cells. Both PB and tissue Vδ1 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 Vδ1 TCR via CD1d-presenting molecules.[39,40] In addition to the CD1 family, human intestinal epithelial Vδ1 T cells respond to stress-induced MICA/B through the synergistic actions of TCR and NKG2D.[41,42] Specifically, in a manner analogous to Vγ9Vδ2 T cells, Vδ1 T cells respond to tumor cells by overexpressing MICA/B and ULBPs via NKG2D.[43,44] Moreover, Vδ1 T cells are activated by the superantigen SE but respond exclusively to SEB rather than SEA.[45] A unique feature of Vδ1 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-Vδ1 AND NON-Vγ9Vδ2 γδT CELLS

Human Vδ3 T cells compose the majority of non-Vδ1 and non-Vγ9Vδ2 γδT cells and are found in healthy PB, the liver[48] and in patients with cytomegalovirus (CMV) infection,[49] HIV infection[50] and B-cell leukemia.[51] Vδ3 T cells, paired with Vγ2 or Vγ3,[50] respond to CD1d and express the degranulation marker CD107a.[52] A Vγ4Vδ5+ T-cell clone has been reported to recognize stressed human cells via TCR binding to endothelial protein C receptor.[53] Furthermore, Vδ4, Vδ6, Vδ7 and Vδ8 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 γδT-cell subsets are highlighted in Table 1.

FUNCTIONAL SUBSETS AND γδT-CELL POLARIZATION

γδT cells share pleiotropic functions with conventional αβ T cells.[55] Each functional subset is induced through the

Table 1 Structural subsets of human γδT cells

Structural subset	*Paired Vγ gene usage*	*Distribution*	*Activation stimulus and/or γδTCR ligands*	*References*
Vδ1	Vγ2/3/4/5/8/9	PB, skin, gut, spleen, liver	MICA/B; ULBPs; B7-H6; CD1c; CD1d; SEB	35,39,44–46
Vδ2	Vγ9	PB	Phosphoantigens; F1-ATPase; BTN3A1; hMSH2; MICA/B; ULBPs; SEs; TSST-1; Nectin-like-5;	20,24,27–28,31–33
Vδ3	Vγ2/3	PB, liver	CD1d	50,52
Vδ5	Vγ4	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 γδT cells by different polarization factors *in vitro*.[56]

IFN-γ-PRODUCING γδT CELLS

Human circulating γδT 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 γδT 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 γδT-cell differentiation toward cytotoxic IFN-γ-producing subsets in the absence of TCR activation.[61]

ANTIGEN-PRESENTING γδ T CELLS

γδT cells also display functional plasticity in terms of indirect anti-infection or antitumor responses.[62,63] Bovine γδT cells present antigens to CD4+ αβT cells.[64] Microbial infections induce professional antigen-presenting cell (APC) functions of human tonsillar γδT 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+ αβT cells.[65] Furthermore, γδT-APCs process soluble protein for cross-presentation on MHC-I and induce CD8+ αβT-effector cell responses more efficiently than monocyte-derived dendritic cells (DCs).[66]

FOLLICULAR B HELPER γδT 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 γδT cells, thus resulting in a T_{FH}-associated phenotype. The transcriptional suppressor Bcl-6 is an indispensable regulator of T_{FH} lineage commitment.[67] γδT_{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-21 R, CD244, CXCL10 and CXCL13, which, in maturing B cells, facilitate the production of high-affinity antibodies against foreign antigens.[68,69]

REGULATORY γδT CELLS

γδT cells also exert immunosuppressive and regulatory activities during immune responses. Casetti *et al.*[70] have reported the induction of Foxp3+ regulatory γδT (γδTreg) cells by TGF-β1 and IL-15, accompanied by antigen stimulation, which inhibits the proliferation of anti-CD3 and anti-CD28 antibody-stimulated PBMCs. Indeed, *in vitro*-expanded Vδ1 T cells stimulated by an anti-human TCR Vδ1 antibody with TGF-β1 predominantly express Foxp3, CD25, glucocorticoid-induced TNFR family-related protein and CTLA4, all of which suppress CD4+ T cell proliferation.[71] Tumor-infiltrating γδTreg cells are induced by IP-10 secreted by breast cancer cells, thereby suppressing T-cell responses and DC maturation.[72] These regulatory γδT 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 γδTreg subset exhibiting CD39 expression that accounts for 60% of γδT17 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+ γδTreg cells suppress the activity of human CD3+ T cells in an adenosine-dependent manner (unpublished data).

IL-17-PRODUCING γδT CELLS

γδT17 cells broadly participate in inflammatory responses, having pathogenic roles during infection and autoimmune diseases.[74] Differentiation into γδ17 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-1β, TGF-β, IL-6 and IL-23.[75] Fresh human cord blood γδT 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-1β and TGF-β are required to generate γδT17 cells in neonates.[77] In addition, IL-23 is highly important for γδT17 cell maturation and growth.[78] In a previous study, we have identified that γδT17 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 γδT-cell subsets with distinct functions.

Table 2 Functional subsets of human γδT cells

Functional subsets	*Polarization*	*References*
IFN-γ-producing γδT	IPP+IL-12+IL-4 antibody; IL-2+IL-21; nonpeptide antigens+IL-21; IL-2+IL-15	57–61
IL-4-producing γδT	IPP+IL-4+IL-12 antibody	57
γδT-APC	Microbial product	6
$γδT_{FH}$	IL-21; HMB-PP+IL-21	56,68
γδTreg	TGF-β+IL-15; Vδ1 TCR antibody+ TGF-β1; IP-10	70–73
γδT17	IL-7+TCR agonists; IL-23; phosphoantigens+IL-1β+TGF-β+IL-6+IL-23; IL-6+IL-1β+TGF-β	14,75–77

Abbreviations: APC, antigen-presenting cell; γδTreg, regulatory γδT; 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.

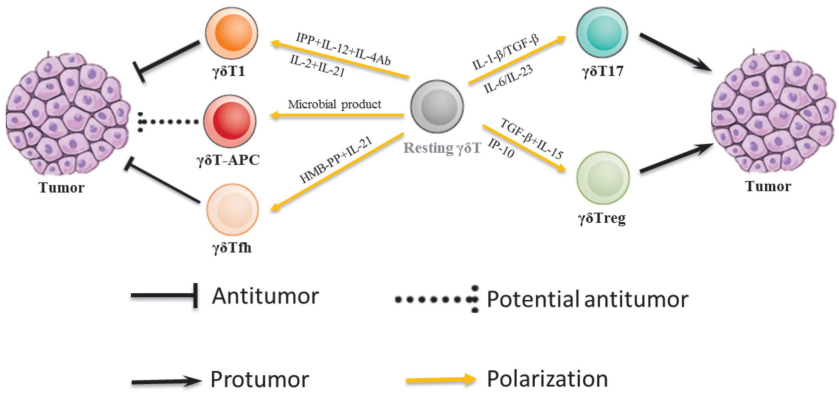

Figure 1 Polarization and responses of human γδT-cell subsets to tumors.

THE ROLE OF γδT-CELL SUBSETS IN TUMOR IMMUNITY

Differentially polarized γδT-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 γδT cells described a potential association between the increased frequency of γδT cells and improved disease-free survival of leukemia patients who received αβT-cell-depleted bone marrow transplants.[79] Recently, intratumoral γδT cells have been demonstrated to be the most significant predictors of favorable survival across various cancer types.[80] γδT cells display cytotoxicity against hematopoietic and solid tumors in an MHC-independent manner.[8] Although their activation mechanisms differ, both Vδ2 and Vδ1 subsets exert potent antitumor effects.[8] One common γδT-cell-mediated killing pattern involves tumor cell recognition via receptor–ligand interactions. TCR is strongly implicated in controlling Vγ9Vδ2 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 Vγ9Vδ2 T-cell cytotoxicity against hemopoietic and epithelial tumors.[11,30,82–84] Vγ9Vδ2 T cells are induced to produce IFN-γ and kill hepatocellular carcinoma cells via the interaction of DNAM-1 and nectin-like-5.[31] γδT cells also exhibit strong cytotoxicity against myeloma cells via NKp44.[85] Furthermore, CD56+ γδT 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, γδT 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+ Vγ9Vδ2 T cells recognize monoclonal antibody-coated lymphoma, chronic lymphocytic leukemia (CLL) and breast cancer cells via CD16 and exert ADCC-dependent cytotoxicity. γδT cells mediate ADCC against B-lineage acute lymphoblastic leukemia via CD19 antibodies.[88] In several other studies, γδT 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, γδT cells have antitumor roles by modulating other effector cells. For instance, Vγ9Vδ2 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] Vγ9Vδ2 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 Vγ9Vδ2 T cells induce NK cells to recognize and kill tumors that are usually resistant to NK cytolysis by increasing NKG2D expression on their surface through CD137L co-stimulation.[93] Phosphoantigen-activated APC-like Vγ9Vδ2 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 Vγ9Vδ2 T cells exert antitumor effects primarily through direct killing, ADCC-dependent cytolysis and by regulating the functions of other innate and adaptive immune cells.

The dramatic expansion of Vδ1 T cells, which usually compose a minor proportion of PB γδT cells, has been observed in solid organ transplant recipients who had developed CMV infection,[95,96] and the long-term expansion of effector Vδ1 T cells is a specific blood signature of CMV infection.[97] Anti-CMV-reactive Vδ1 T cells recognize intestinal tumor epithelial cells. After recognition, Vδ1 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 Vδ1 T cells demonstrate better antitumor potential and are associated with reduced cancer risk in kidney transplant recipients.[99] Expanded Vδ1 T cells expressing CD8αα after CMV reactivation after allogeneic stem cell transplantation recognize both CMV-infected cells and primary leukemic blasts.[100] In contrast, *ex vivo*-expanded Vδ1 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 Vδ1 T cells, although the mechanism underlying the recognition of CMV-infected cells and tumor cells by Vδ1 T cells requires further study.

In addition to CMV-associated antitumor activity, both circulating and tumor-infiltrating Vδ1 T cells respond to malignancies of hematological and epithelial origin. Circulating Vδ1 T cells contribute to the antitumor response against low-grade non-Hodgkin lymphoma (NHL) by recognizing ULBPs on lymphoma cells.[44] Moreover, Vδ1 T cells, but not Vγ9Vδ2 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 Vδ1 T cells demonstrate more potent killing of colon cancer cells than Vγ9Vδ2 T cells via cytolytic receptor–ligand interactions.[13] Moreover, human Vδ1 T cells have been reported to inhibit tumor metastases independently of primary tumor control in a xenograft model of colon cancer.[104] Tumor-infiltrating Vδ1 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 Vδ1 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 Vδ1 T cells isolated from various solid tumors demonstrate stronger cytotoxicity against tumor cell lines and/or freshly isolated tumor cells compared with Vγ9Vδ2 T cells.[105,108–112] Notably, in a previous study, the majority of Vδ1 T-cell lines exerted robust cytotoxic responses against the melanoma cell line A375, whereas only two of eight Vδ2 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 γδT cells exert antitumor effects, Vδ1 T cells are potentially better killers than Vγ9Vδ2 T cells, at least in the context of certain tumors.

PROTUMOR EFFECTS

Although γδT cells demonstrate potent antitumor capacity, paradoxically they also exert protumor effects by promoting noncytotoxic inflammation and regulatory functions that subvert cytotoxic antitumor immunity. Intratumoral γδT cell numbers are positively associated with advanced tumor stages and are inversely correlated with breast cancer prognosis.[113] γδT 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, γδT17 cells exert tumor-promoting effects in mice by facilitating angiogenesis.[114,116] γδT17 cells also promote breast cancer metastasis because mice treated with γδT-cell-depleting agents or anti-γδTCR antibodies are profoundly protected against pulmonary and lymph node metastases.[117] However, there have been few studies investigating the role of human γδT17 cells in tumor immunity. In our previous study, we have found that tumor-infiltrating γδT17 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, γδT17 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, γδT17 cells have been found to accumulate during later stages of tumor progression.[118] We have also demonstrated a positive correlation between γδT17 cell numbers and advancing tumor stages of human colorectal cancer.[14]

γδT cells possess potential regulatory roles in the control of tumor immune responses. For example, according to Peng *et al.*,[73] tumor-infiltrating γδT 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, γδTreg 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 γδTreg subset in human colorectal cancer that promotes an immunosuppressive microenvironment via a metabolism-related mechanism (unpublished data). Thus, certain γδT cell subsets behave as immunosuppressive cells and promote tumor progression in specific cancers. However, more studies focusing on the polarization mechanisms of protumor γδT cells in human tumor microenvironments (TMEs) are needed.

CLINICAL IMPLICATIONS

Given their potent MHC-unrestricted antitumor effector activities, γδT cells are attractive candidates for antitumor immunotherapies. The cytotoxic features of the Vδ1 and Vδ2 subsets have been investigated.[8] Preclinical and clinical studies have paved the way for Vγ9Vδ2 T-cell-mediated immunotherapy, given the high-frequency and broad antitumor properties of this cell type.[120] Clinical-scale expansion of Vγ9Vδ2 T cells via direct stimulation by phosphoantigens or the induction of agonist accumulation with aminobisphosphonates makes Vγ9Vδ2 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 Vδ1 subsets in basic research,[13,121,122] Vδ1 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 Vδ1 T cells and simultaneously inhibits Vδ2 T-cell growth,[123] thus providing a novel basis to develop preferential expansion approaches for Vδ1 T cells. Recently, Almeida *et al.*[124] have reported a robust two-step protocol for the selective expansion of Vδ1 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 Vδ1 T cells to move forward with autologous or allogeneic cell therapies for both hematological and solid tumors.

Immunosuppressive functions of γδT cells infiltrating breast cancer and colorectal cancer TMEs have been described.[14,73] The emerging evidence supporting protumor roles for specific γδT-cell subsets potentially poses an obstacle to the development of future therapies.[125] Although knowledge of γδT-cell function in the TME has gradually increased, it remains a challenge to determine whether the inflammatory and regulatory features of γδT 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 γδT cells before transfer or to combine γδT-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 γδT cells, and it is possible to polarize Vδ2 T cells into nearly all functional subsets. However, efforts should be made to further distinguish between Vδ1 and Vδ2 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 γδT-cell type has been reported. A myriad of evidence indicates either antitumor effects or tumor-promoting activities for γδT cells in tumor immunity. The dual role of γδT cells is closely associated with their complex surrounding microenvironment, which influences γδT-cell polarization. Our group has identified the ability of *ex vivo*-expanded Vδ1 T cells to exert favorable killing activity against colon cancer, whereas γδT17 cells in colon cancer tissue, the majority of which demonstrate Vδ1 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 γδT-cell subsets should elucidate their effects in tumor immunity, thus providing sufficient evidence for the application of γδT-cell subsets for antitumor adoptive immunotherapy or for targeting certain inflammatory or regulatory γδT-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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