

Review Article



Heterogeneity of Human $\gamma\delta$ T Cells and Their Role in Cancer Immunity

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Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

BTN3A, butyrophilin 3A; DETC, dendritic epidermal T cell; KIR, killer inhibitory receptor; NCR, natural cytotoxicity receptor; NKG2D, NK group 2 member D; Tfh, follicular Th; TME, tumor microenvironment; ULBP, UL16-binding protein

ABSTRACT

The $\gamma\delta$ T cells are unconventional lymphocytes that function in both innate and adaptive immune responses against various intracellular and infectious stresses. The $\gamma\delta$ T cells can be exploited as cancer-killing effector cells since $\gamma\delta$ TCRs recognize MHC-like molecules and growth factor receptors that are upregulated in cancer cells, and $\gamma\delta$ T cells can differentiate into cytotoxic effector cells. However, $\gamma\delta$ T cells may also promote tumor progression by secreting IL-17 or other cytokines. Therefore, it is essential to understand how the differentiation and homeostasis of $\gamma\delta$ T cells are regulated and whether distinct $\gamma\delta$ T cell subsets have different functions. Human $\gamma\delta$ T cells are classified into V δ 2 and non-V δ 2 $\gamma\delta$ T cells. The majority of V δ 2 $\gamma\delta$ T cells are V γ 9 δ 2 T cells that recognize pyrophosphorylated isoprenoids generated by the dysregulated mevalonate pathway. In contrast, V δ 1 T cells expand from initially diverse TCR repertoire in patients with infectious diseases and cancers. The ligands of V δ 1 T cells are diverse and include the growth factor receptors such as endothelial protein C receptor. Both V δ 1 and V δ 2 $\gamma\delta$ T cells are implicated to have immunotherapeutic potentials for cancers, but the detailed elucidation of the distinct characteristics of 2 populations will be required to enhance the immunotherapeutic potential of $\gamma\delta$ T cells. Here, we summarize recent progress regarding cancer immunology of human $\gamma\delta$ T cells, including their development, heterogeneity, and plasticity, the putative mechanisms underlying ligand recognition and activation, and their dual effects on tumor progression in the tumor microenvironment.

Keywords: T-lymphocyte subsets; $\gamma\delta$ T cell; T Cell Receptors, gamma delta; Tumor microenvironment

INTRODUCTION

Among 3 main lineages of lymphocytes— $\alpha\beta$ T cells, $\gamma\delta$ T cells, and B cells, $\gamma\delta$ T cells are the most enigmatic lymphocytes that express TCRs rearranged from TCR γ and δ genes (1-3). The $\gamma\delta$ T cells are one of the innate immune cells that have a pivotal role in cancer immunosurveillance as the deficiency of $\gamma\delta$ T cells increased the susceptibility to cancers (4-7). They can mediate potent direct cytotoxicity by recognizing transformed target cells via the $\gamma\delta$ TCRs, but they may also detect cancer cells via activating NK cell receptors such as NK

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group 2 member D (NKG2D) or natural cytotoxicity receptors (NCRs) (8,9). The application of $\gamma\delta$ T cells, mostly $V\gamma 9V\delta 2$ T cells, for cancer immunotherapy has been explored against various tumors of hematological and epithelial origin (10-16). Many clinical trials have shown that those treatments are feasible and safe, but with some obvious limitations (4,12,17). Therefore, a better understanding of $\gamma\delta$ T cell subset-specific responses during tumor immunity is vital to rationally develop optimal strategies for maximizing the anti-tumor activity of $\gamma\delta$ T cells and inhibiting their pro-tumor activity. Here, we summarize the recent progress regarding the immunobiology of human $\gamma\delta$ T cells, including the heterogeneity and plasticity, the putative mechanisms of ligand recognition and activation, their positive and negative effects on the cancer progression, and the future perspective of immunotherapy using $\gamma\delta$ T cells.

ORIGIN AND DEVELOPMENT OF THE HUMAN $\gamma\delta$ T CELLS

The $\gamma\delta$ T cells develop earlier than $\alpha\beta$ T cells in the thymus and are exported to different peripheral tissues according to the chronological order of the thymic development (18,19). In the mouse, the first $\gamma\delta$ T cells at the embryonic day of 14, $V\gamma 5^+V\delta 1^+$ T cells are selected by selection and upkeep of intraepithelial T cells protein 1 presented on thymic epithelial cells and become dendritic epidermal T cells (DETCs) responsible for body-barrier surveillance (20,21). A later process of thymic T cell development generates $V\gamma 6^+V\delta 1^+$ T cells that are destined for the female genital tract, peritoneal cavity and tongue, and other $\gamma\delta$ T cells with diverse VDJ clonotypes containing $V\gamma 1, 2, 4,$ and 7 segments (18,22). Whereas the early developing $\gamma\delta$ T cells have invariant TCRs, the $\gamma\delta$ T cells appearing during the later period of the development are diverse in TCR repertoire (22-24).

Human $\gamma\delta$ T cells are also present in the thymus as well as the periphery, suggesting the thymic development of human $\gamma\delta$ T cells (25). Although adult blood $\gamma\delta$ T cells are predominated by $V\gamma 9V\delta 2$ cells, neonatal cord blood $\gamma\delta$ T cells express a diversity of $V\gamma$ and $V\delta$ chains paired in various combinations, and the majority of neonatal $\gamma\delta$ T cells are $V\gamma 9^+V\delta 1^+$ cells (26,27). Therefore, the adult blood $V\gamma 9V\delta 2$ cells appear to represent the post-natal expansion of $V\gamma 9V\delta 2$ cells expressing canonical CDR3s in response to microbial phosphoantigens that are described below (28-30). Human $V\gamma 9V\delta 2$ cells have been shown to expand rapidly after birth within 1 year of life (31). In the adult, $V\delta 1$ and $V\delta 2$ $\gamma\delta$ T cells are localized in the barrier tissues and the peripheral blood, respectively (32).

HETEROGENEITY OF THE HUMAN $\gamma\delta$ T CELLS

Although $\gamma\delta$ T cells are cousins of $\alpha\beta$ T cells, $\gamma\delta$ T cells directly recognize Ags via their $\gamma\delta$ TCRs without the need of MHC molecules similarly to B cells (1,33,34). The $\gamma\delta$ T cells are sometimes referred to as innate lymphocytes since they can recognize microbial or stress-induced patterns and respond rapidly without previous exposure to the Ags (1,24). However, some $\gamma\delta$ T cells exhibit highly adaptive features such as clonal expansion and differentiation from naïve cells to effector cells (35). The overall characteristics of $\gamma\delta$ T cells may be positioned between NK cells and $CD8^+$ T cells (36). The $\gamma\delta$ T cells are heterogeneous concerning functional features depending on the usage of TCR γ and δ chains and the tissue localization. The $\gamma\delta$ T cell population consists of tissue-resident and peripheral blood $\gamma\delta$ T cells (1). Human $\gamma\delta$ T cells account for 0.5%–5% of all peripheral blood T cells (2,7).

In humans, several functional $V\gamma$ gene segments (including $V\gamma 2$, $V\gamma 3$, $V\gamma 4$, $V\gamma 5$, $V\gamma 8$, $V\gamma 9$, and $V\gamma 11$) rearrange into 5 $J\gamma$ segments and 2 $C\gamma$ segments on chromosome 7 to generate TCR γ chains, whereas TCR δ chains are generated by the rearrangement of at least 7 $V\delta$, 3 $D\delta$, 3 $J\delta$, and 1 $C\delta$ segments on chromosome 14 (2,3,7,37). Whereas $V\delta 1$, $V\delta 2$, and $V\delta 3$ segments are used only in the rearrangement of the TCR δ chains, $V\delta 4$ – $V\delta 7$ segments are also used in the rearrangement of the TCR α chains and have alternative gene names belonging to TCR $V\alpha$ gene segments (37). The functional features of $\gamma\delta$ T cells are closely correlated with the usage of the TCR δ chains (24). Among 7 $V\delta$ segments, $V\delta 1$ and $V\delta 2$ segment-using $\gamma\delta$ TCRs are the most common human $\gamma\delta$ TCRs (2).

As the most abundant human $\gamma\delta$ T cells are $V\gamma 9V\delta 2$ T cells that recognize unique phosphoantigens and $V\delta 1$ T cells have adaptive features distinct from $V\gamma 9V\delta 2$ T cells, $\gamma\delta$ T cells are commonly classified into $V\delta 2$ and non- $V\delta 2$ $\gamma\delta$ T cells (2,35,38). The $V\gamma 9V\delta 2$ T cells are the most well-known human $\gamma\delta$ T cells and have been exploited for anti-cancer immunotherapy (10,11). The characteristics and the adaptive features of non- $V\delta 2$ $\gamma\delta$ T cells, especially $V\delta 1$ $\gamma\delta$ T cells, are recently recognized, and these $V\delta 1$ $\gamma\delta$ T cells are also thought to be a candidate for anti-cancer immunotherapy (35).

THE $\gamma\delta$ TCR STRUCTURE AND ACTIVATION OF THE HUMAN $\gamma\delta$ T CELLS

Although $\gamma\delta$ T cells share TCR rearrangement mechanism and memory functions with $\alpha\beta$ T cells, they differ in the immune response kinetics and mechanisms of target cell recognition (39). The $\gamma\delta$ T cells do not recognize MHC molecules, but many $\gamma\delta$ T cells respond to non-peptide Ags or MHC-like molecules, such as MHC class I-related chain A (MICA), MICB, or UL16-binding protein (ULBP), that are upregulated in cells under stressed conditions such as infection or cancer transformation in MHC-unrestricted manner (2,3). Similarly to $\alpha\beta$ TCRs, $\gamma\delta$ TCRs are also associated with CD3 molecules, but differently from murine $\alpha\beta$ TCRs, murine $\gamma\delta$ TCRs contain only CD3 $\gamma\epsilon$ dimers, not CD3 $\delta\epsilon$ dimers (40). Notably, murine $\gamma\delta$ TCR cells can develop in the absence of CD3 ϵ or CD3 δ (41,42), but the expression of CD3 γ is indispensable for the murine $\gamma\delta$ T cell development (43). Furthermore, CD3 ζ chain is not necessary for the $\gamma\delta$ T cell development and Fc ϵ RI γ chain, a CD3 ζ chain family member that can dimerize with CD3 ζ , is expressed upon activation and then included in the $\gamma\delta$ TCR complexes (44). On the other hand, human $\gamma\delta$ TCR complex contains CD3 δ chain and shows a TCR $\gamma\delta$ CD3 $\epsilon_2\delta\gamma\zeta_2$ stoichiometry similarly to human $\alpha\beta$ TCR complex, whereas mouse $\gamma\delta$ TCR complex has a TCR $\gamma\delta$ CD3 $\epsilon_2\gamma_2\zeta_2$ stoichiometry (45). Human $\gamma\delta$ TCR signaling is less dependent on CD3 γ chain than CD3 δ chain as human patient lacking CD3 γ have abundant peripheral blood $\gamma\delta$ T cells expressing high levels of $\gamma\delta$ TCR (46). Interestingly, forced expression of human, but not murine, CD3 δ transgene rescue the $\gamma\delta$ T cell development in mice deficient in both CD3 δ and CD3 γ genes, suggesting the unique role of human CD3 δ in the TCR signaling (45).

The $\gamma\delta$ TCR signaling is qualitatively different from the $\alpha\beta$ TCR signaling (44). The $\gamma\delta$ TCRs self-oligomerize and cause constitutive signaling in the absence of ligands (47). These $\gamma\delta$ TCR signaling characteristics are similar to those of pre- $\alpha\beta$ TCR signaling responsible for the β selection during thymic T cell development (48). During the thymic $\gamma\delta$ T cell development, $\gamma\delta$ T cells that encounter strong agonistic ligands obtain the capability of secreting IFN- γ . In contrast, $\gamma\delta$ T cells that do not encounter strong agonists adopt IL-17-default position (1,47).

In the periphery, the stimulation of $\gamma\delta$ T cells via $\gamma\delta$ TCR and costimulatory receptors or NK cell receptors triggers $\gamma\delta$ T cells to undergo clonal expansion and differentiation into effector cells and to produce large quantities of pro-inflammatory cytokines such as IFN- γ or IL-17. Upon activation, $\gamma\delta$ T cells can also exert a potent cytotoxic activity without the obligatory delay associated with clonal expansion and differentiation (49).

Although $\gamma\delta$ TCR is regarded as an activating receptor, $\gamma\delta$ TCR may act as an inhibitory receptor in certain contexts. The consequence of the constitutive $\gamma\delta$ TCR signaling can be inhibition of $\gamma\delta$ T cell activation when the ligands on target cells are constitutively presented (50). In NK cells, the constitutive inhibitory signaling through killer inhibitory receptor (KIR) sets up a threshold that NK cells are not easily activated, and a full activation of NK cell requires very high concentrations of activating ligands for NK cell-activating receptors and/or downregulation of inhibitory ligand, MHC class I on the target cells (51). The V γ 5 δ 1 TCRs in mouse DETCs form constitutive immunological synapses with keratinocytes in the steady state and are argued to have a role similar to KIR on NK cells (20).

Since $\gamma\delta$ T cells have a lot of NK cell-activating receptors such as NCRs and NKG2D (8,9), the functional roles of $\gamma\delta$ TCR should be carefully investigated in heterogeneous subpopulations of $\gamma\delta$ T cells since the NK receptors, not $\gamma\delta$ TCR, could be main receptors for $\gamma\delta$ T cell activation. The NK cell-activating receptors can be considered as costimulatory receptors if $\gamma\delta$ TCR and NK cell receptors induce synergistic signaling for $\gamma\delta$ T cell responses (52). The list of costimulatory receptors for $\alpha\beta$ T cells has been expanded and includes a prototype costimulatory molecule CD28 (53). The relevance of costimulatory molecules for $\alpha\beta$ T cells in $\gamma\delta$ T cells remains debatable. About 40%–60% of $\gamma\delta$ T cells express CD28, and the expression of CD28 is decreased upon the activation of $\gamma\delta$ T cells (54,55). Since anti-CD28 agonistic Abs enhance human $\gamma\delta$ T cell proliferation, the role of CD28 as a costimulatory molecule is valid in a subpopulation of human $\gamma\delta$ T cells. Considering the phenotypes of memory and effector CD8⁺ $\alpha\beta$ T cells (56), it may be hypothesized that the expression of CD28 is lost upon the prolonged activation of a subpopulation of $\gamma\delta$ T cells. It is noteworthy that a higher proportion of V δ 1 $\gamma\delta$ T cells do not express CD28 than that of V δ 2 $\gamma\delta$ T cells, but the most of V δ 2 $\gamma\delta$ T cells express CD28 similarly to naïve $\alpha\beta$ T cells (35,57).

THE $\gamma\delta$ T CELLS IN THE TUMOR MICROENVIRONMENT (TME)

Recruitment of human $\gamma\delta$ T cells into the TME

Cancer is characterized not only by transformed cancer cells but also by non-cancer cells, such as immune cells, fibroblasts, and endothelial cells, and the extracellular matrix that establishes the TME. Initially, the cellular stresses experienced by transformed cancer cells trigger the upregulation of ligands for NK cell receptors (58). Although initially recruited NK cells can kill cancer cells, the cytotoxic activity of NK cells is not sustained but exhausted when cancer cells outnumber NK cells in the advanced stage of cancer (59). The persistent chronic inflammation associated with cancer recruits many kinds of immune cells, including Treg cells and myeloid-derived suppressor cells into the TME. It is a common consensus that the TME inhibits the anti-tumor immune responses in most clinical situations (60-62).

The $\gamma\delta$ T cells also infiltrate into a variety of the tumors in the early and late stages of cancer development, where they are known to modulate the anti-tumor response through pro- or

anti-inflammatory cytokines and their interactions with different types of innate and adaptive immune cells in the TME (7,49,63,64). The $\gamma\delta$ T cells migrate into the TME in response to CC chemokines such as MCP-1, regulated on activation normal T cell expressed and secreted, MIP-1 α and MIP-1 β (11,35,65).

Major tumor-infiltrating $\gamma\delta$ T cell subsets: human V δ 1 and V γ 9V δ 2 T cells

In humans, V δ 1 and V γ 9V δ 2 T cells are 2 main populations of $\gamma\delta$ T cells in the tissues and peripheral blood. In tumors, one subset can be predominant over the other depending on the types and origin of the tumors (7,11,49,64-67). Both V δ 1 and V γ 9V δ 2 T cells have the cytotoxic capability and can have anti-cancer activity (11,36). The 2 subsets of $\gamma\delta$ T cells express distinct chemokine receptors and cell adhesion molecules, suggesting different homing mechanisms that can be selectively utilized for cancer immunotherapy (35,68,69). A diagram is displayed in Fig. 1, which shows their differential involvement in the anti-cancer immunity.

The human V γ 9V δ 2 T cells are the most predominant $\gamma\delta$ T cells in the adult peripheral blood, but they are not a major $\gamma\delta$ T cell population at the time of birth as the V δ 1 $\gamma\delta$ T cells are predominant during fetal and early life (24,31). The V γ 9V δ 2 T cells expand postnatally in response to phosphoantigens by microbes. The canonical V γ 9V δ 2 T cells with V γ 9J γ P sequences recognize phosphoantigens presented by butyrophilin 3A (BTN3A). Interestingly, prenyl pyrophosphates (phosphoantigens) bind to the intracellular B30.2 domain of BTN3A1,

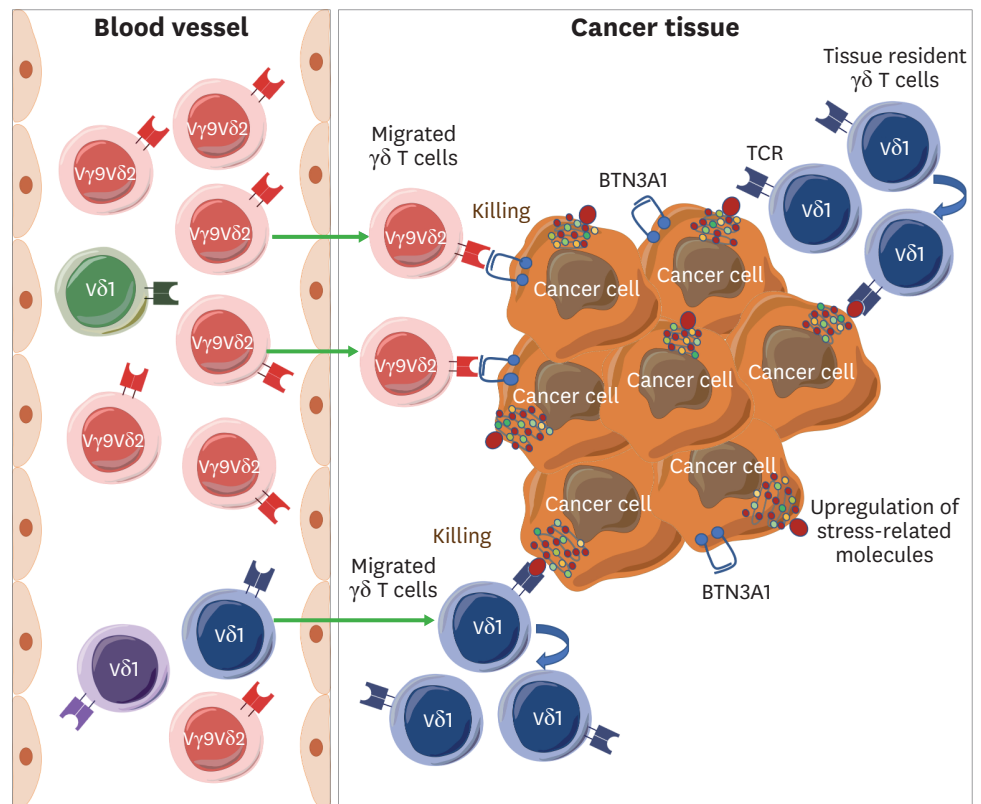


Figure 1. Differential recruitment of V δ 1 and V γ 9 δ 2 $\gamma\delta$ T cells into the tumor tissue. In blood, V γ 9 δ 2 $\gamma\delta$ T cells are predominant over V δ 2 $\gamma\delta$ T cells in healthy individuals. Most of the V γ 9 δ 2 $\gamma\delta$ T cells have canonical TCRs responding to prenyl pyrophosphates that are elevated in cancer cells and are recruited into the tumor via chemokine receptors. In contrast, some clonotypes of V δ 1 $\gamma\delta$ T cells are selected from a diverse V δ 1 TCR repertoire. Specific V δ 1 $\gamma\delta$ T cells migrate into the tumor tissues, expand, and kill cancer cells. The tissue-resident V δ 1 $\gamma\delta$ T cells may respond to the tissue stress and proliferate to kill cancer cells.

suggesting that the V γ 9V δ 2 TCR senses the internal changes of mevalonate or non-mevalonate metabolic pathways within cancer or infected cells, respectively (70,71). Elevated cytoplasmic prenyl pyrophosphate levels as a result of a dysregulation of the mevalonate pathway triggers an inside-out signaling leading to a structural change of the extracellular domain of BTN3A1, which enhances the binding force between BTN3A1 and V γ 9V δ 2 TCR. It is noteworthy that the RhoB activation in cancer cells is another determinant for the relocalization of BTN3A1 and the activation of V γ 9V δ 2 $\gamma\delta$ T cells (72). Therefore, the canonical V γ 9V δ 2 $\gamma\delta$ T cells can be cancer-killing lymphocytes through the recognition of the altered metabolism of cancer cells and the mutation of RhoB. However, the functional role and Ags of non-canonical V γ 9V δ 2 $\gamma\delta$ T cells are not well understood. The non-canonical V γ 9V δ 2 $\gamma\delta$ T cells predominate over the canonical V γ 9V δ 2 $\gamma\delta$ T cells in the fetal tissues, but the canonical V γ 9V δ 2 $\gamma\delta$ T cells become predominant in the adult blood. Interestingly, glioblastoma-infiltrating $\gamma\delta$ T cells have non-canonical TCR repertoire using C γ 2 segment (66). Regarding the ligands for V γ 9V δ 2 $\gamma\delta$ TCR, it is needed to address the role of a novel ligand for V γ 9 TCR, BTN2A1, in the cancer immunity and whether BTN2A1 is upregulated in the cancer cells (73). The V γ 9V δ 2 T cells also recognize cancer cells through stress-related proteins that can be upregulated upon malignant transformation, including the F1-ATPase, MICA/B, heat shock protein 60, ULBP, human MutS homolog 2, and DNAX-associated molecule-1 through NK cell receptors (1,2,74,75).

Whereas most of the V γ 9V δ 2 T cells have restricted canonical TCRs, the V δ 1 T cells have diverse repertoires that use various kinds of V γ chains (35). In most adults, a small number of specific clonotypes emerge from an initially unfocused neonatal V δ 1 TCR repertoire, undergo pronounced clonal expansion, and ultimately dominate the V δ 1 T cell compartment (69). The TCR-diverse CD27^{high} and highly TCR focused CD27^{low/-} populations represent naïve and effector V δ 1 T cell subsets, respectively. The transition from naïve to effector V δ 1 T cells is accompanied by a switch from lymphoid to peripheral homing receptors. Although ligands detected by V δ 1 TCRs remain largely uncharacterized, some peripheral circulating and tissue-resident V δ 1 T cells recognize CD1c, the lipid-presenting MHC-like molecule CD1d, or MHC-related protein 1 (2,76-78). In addition to the TCR, V δ 1 T cells also respond to a distinct set of cellular stress signals expressed by cancerous cells, such as MICA/B, ULBPs, B7-H6, and BAT3 via NKG2D or NCRs (1,77,79). In many tumors, V δ 1 $\gamma\delta$ T cells are predominant over the V γ 9V δ 2 $\gamma\delta$ T cells, which suggests that the V δ 1 $\gamma\delta$ T cells expand responding to cancer Ags (80). The utilization of the V δ 1 $\gamma\delta$ T cells is a promising option for cancer immunotherapy. Therefore, the relative importance of V δ 1 $\gamma\delta$ T cells and V γ 9V δ 2 $\gamma\delta$ T cells should be considered in the future immunotherapy using $\gamma\delta$ T cells (4,10,12,14,16). Non-V δ 1 non-V γ 9V δ 2 $\gamma\delta$ T cells should also be considered as the V γ 4V δ 5 $\gamma\delta$ T cells are able to eliminate cancer cells by recognizing endothelial protein C receptor (81).

The $\gamma\delta$ T cells have both anti-tumor and pro-tumor activities

Upon migration to the TME, $\gamma\delta$ T cells exert potent anti-tumor effects via multiple mechanisms (77,82). The $\gamma\delta$ T cells can eliminate cancer cells via cytolytic receptor-ligand interactions including Fas ligand (83) and TNF-related apoptosis-inducing ligand (84) in addition to granzyme B and perforin and also have cytostatic anti-cancer activities by releasing IFN- γ or TNF- α (82,85). The $\gamma\delta$ T cells are also able to kill Ab-coated cancer cells by Ab-dependent cellular cytotoxicity using cell surface CD16 (Fc γ RIII) similar to NK cells (86). Lastly, $\gamma\delta$ T cells exhibit indirect anti-tumor responses by modulating different immune cell types including DCs, NK cells, neutrophils, $\alpha\beta$ T cells and B cells in the TME (1,4,22,75,77,82,85).

In general, the extent of intratumoral $\gamma\delta$ T cell infiltration is highly associated with the CD8⁺ T cell signature and patients' prognosis, suggesting that $\gamma\delta$ T cells largely perform anti-tumor

activity rather than pro-tumor activity (87,88). However, complex interactions between TME and intratumoral $\gamma\delta$ T cells can result in the diversion of anti-tumor $\gamma\delta$ T cells into pro-tumor cells. Therefore, the precise role of $\gamma\delta$ T cells in each individual patient may depend on the specific $\gamma\delta$ T cell subsets and their functional polarization in the TME (63,77,82,85). Regarding T cell polarization, it is generally stated that Th1 and follicular Th (Tfh) cells have anti-tumor activity, whereas Th17 and Treg cells have pro-tumor activity (89). As the $\gamma\delta$ Tfh cell-driven GC response tends to induce autoreactive B cells instead of pathogen-specific B cells, the anti-tumor activity of Tfh cells is not well established (90), but the involvement of Tfh cells in cancer tissues indicates an organized anti-tumor immunity with tertiary lymphoid tissue (91). Effector $\gamma\delta$ T cells can also be classified as $\gamma\delta$ Th1, $\gamma\delta$ Th2, $\gamma\delta$ Th17, $\gamma\delta$ Tfh, and $\gamma\delta$ Treg cells based on their functional polarization (1,7,22,35,49,75,77,85,92). Interestingly, in response to different cytokines, $\gamma\delta$ T cells can trans-differentiate from one phenotype to another (1,7,22,35,49,75,77,85,92). Both V δ 1 and V γ 9V δ 2 T cells can be polarized into $\gamma\delta$ Th1 cells, $\gamma\delta$ Tfh cells, $\gamma\delta$ T17 cells, $\gamma\delta$ Treg cells, and $\gamma\delta$ Th2 cells with distinct cytokines. It is important to investigate further whether $\gamma\delta$ T cells are a primary driver of T cell polarization or whether the immunotherapy targeting $\gamma\delta$ T cells can change the overall polarization of $\alpha\beta$ T cells within the TME.

The $\gamma\delta$ T cells are also subjected to immune exhaustion similarly to cancer-reactive cytotoxic T cells and NK cells. Although the nature of the $\gamma\delta$ T cell immune exhaustion is not well reported, prolonged stimulation of $\gamma\delta$ T cells appears to trigger their immune exhaustion. Since the immune exhaustion is reviewed extensively elsewhere (93-95), it will not be discussed here. It would be important and interesting to address how easily and deeply $\gamma\delta$ T cells are exhausted and whether exhausted $\gamma\delta$ T cells can be easily reawakened by strong stimuli, including cytokines or Ags such as phosphoantigens.

FUTURE DIRECTIONS FOR OPTIMIZING ADOPTIVE $\gamma\delta$ T CELL TRANSFER AS AN ALTERNATIVE CANCER IMMUNOTHERAPY

The ability of $\gamma\delta$ T cells to recognize the cellular stress via an MHC-independent mechanism and to potentiate other innate and adaptive immune cells makes them attractive mediators of cancer immunotherapy with potent and broad anti-tumor cytotoxicity (4,7,11,64,65,68,77,82,85,89). Especially, given their potent MHC-unrestricted anti-tumor activities, $\gamma\delta$ T cells also can be considered as universal allogeneic adoptive T cell transfer for cancer patients. Accordingly, recent applications of $\gamma\delta$ T cells to solid tumors have yielded promising results with associated clinical benefits, but issues of limited efficacy still remain with an average response ratio of only 21% and low proportion of complete remissions (7,14-16,85,96,97). Unfortunately, the tumor cells are effectively protected from tumor cell-killing immune activities in the immune-suppressive TME, which may also block the infiltration of infused $\gamma\delta$ T cells. Furthermore, the anti-tumor function of $\gamma\delta$ T cells can be limited by the pleiotropic effects of a mixture of heterogeneous populations of immune cells in the TME (4,7,14,16,63,64,85,97).

Therefore, current efforts in favor of a durable anti-tumor benefit from $\gamma\delta$ T cell immunotherapy lie in the quest to minimize activation-induced $\gamma\delta$ T cell death, anergy, and the polarization to specific $\gamma\delta$ T cells with immunosuppressive function (4,7,14,15,92,98-100). Additionally, several cytokines such as IL-15, IL-18, and IL-21 have been found to have the ability to promote the

expansion of $\gamma\delta$ T cells with a higher proliferative capacity, a more pronounced Th1 polarization, and an increased cytotoxic capacity and secretion of immune-stimulating paracrine factors such as GM-CSF, IFN- γ , and TNF- α (101-103). In particular, disruption of the immunosuppressive TME could be a new strategy for improving the anti-tumor efficacy of $\gamma\delta$ T cells. For example, IL-36 γ acts synergistically with TCR signaling and is able to promote IFN- γ production by CD8⁺ T cells, NK cells, and $\gamma\delta$ T cells by transforming the TME in favor of cancer eradication (104).

Up to date, clinical trials have been based on the adoptive transfer of peripheral circulating V γ 9V δ 2 T cells after *ex vivo* expansion and activation (11,15,16,68,82,97,105). Given the accumulating pieces of evidence supporting the superior anti-tumor functionality of V δ 1 T cells compared with that of V γ 9V δ 2 T cells, at least in the context of certain tumors (14,67,77,98,99,106-110), 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. An interesting property of V δ 1 T cells for the adoptive transfer approach is their CCL2-mediated chemotaxis toward tumors (67,111,112). V δ 1 T cells are also less susceptible to activation-induced cell death and could persist in the circulation for many years, which is in favor of a durable anti-tumor immunity (98,99,110). Intriguingly, IL-4 promotes the proliferation of V δ 1 T cells and simultaneously inhibits V δ 2 T-cell growth (77,80,113), thus providing a novel basis to develop the preferential expansion approaches for V δ 1 T cells.

CONCLUDING REMARKS

Although $\gamma\delta$ T cells are a small population of lymphocytes, they contribute significantly to rapid and sustained immune responses against cancer. In order to utilize the inherent activity of $\gamma\delta$ T cells for cancer immunotherapy, it is critical to better characterize human $\gamma\delta$ T cell subsets and the engaged mechanisms in various types of cancers. It is also necessary to understand the central paradigms that govern the tissue tropism, the stage of differentiation, the activation status, and the immune checkpoint receptor expression in $\gamma\delta$ T cells so that $\gamma\delta$ T cells can be durably activated with a potent anti-tumor phenotype. To maintain the anti-tumor activity of $\gamma\delta$ T cells for a long period of time, the specific depletion of pro-tumor $\gamma\delta$ T cells before the immunotherapy, the co-transfer of other immune cells that activate $\gamma\delta$ T cells, and the modification of the cytokine balance in the TME should be considered in the immunotherapy using $\gamma\delta$ T cells. In summary, as $\gamma\delta$ T cells are heterogeneous, the pro-tumor or anti-tumor activities of different $\gamma\delta$ T cell populations need to be thoroughly delineated and utilized to maximize the efficacy of the immunotherapy using $\gamma\delta$ T cells.

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