Heterogeneity of Human γδ T Cells and Their Role in Cancer Immunity

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ABSTRACT

The γδ T cells are unconventional lymphocytes that function in both innate and adaptive immune responses against various intracellular and infectious stresses. The γδ T cells can be exploited as cancer-killing effector cells since γδ TCRs recognize MHC-like molecules and growth factor receptors that are upregulated in cancer cells, and γδ T cells can differentiate into cytotoxic effector cells. However, γδ 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 γδ T cells are regulated and whether distinct γδ T cell subsets have different functions. Human γδ T cells are classified into Vδ2 and non-Vδ2 γδ T cells. The majority of Vδ2 γδ T cells are Vγ9δ2 T cells that recognize pyrophosphorylated isoprenoids generated by the dysregulated mevalonate pathway. In contrast, Vδ1 T cells expand from initially diverse TCR repertoire in patients with infectious diseases and cancers. The ligands of Vδ1 and Vδ2 γδ T cells are diverse and include the growth factor receptors such as endothelial protein C receptor. Both Vδ1 and Vδ2 γδ 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 γδ T cells. Here, we summarize recent progress regarding cancer immunology of human γδ 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; γδ T cell; T Cell Receptors, gamma delta; Tumor microenvironment

INTRODUCTION

Among 3 main lineages of lymphocytes—αβ T cells, γδ T cells, and B cells, γδ T cells are the most enigmatic lymphocytes that express TCRs rearranged from TCR γ and δ genes (1-3). The γδ T cells are one of the innate immune cells that have a pivotal role in cancer immunosurveillance as the deficiency of γδ T cells increased the susceptibility to cancers (4-7). They can mediate potent direct cytotoxicity by recognizing transformed target cells via the γδ TCRs, but they may also detect cancer cells via activating NK cell receptors such as NK
group 2 member D (NKG2D) or natural cytotoxicity receptors (NCRs) (8,9). The application of γδ T cells, mostly Vγ9Vδ2 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 γδ T cell subset-specific responses during tumor immunity is vital to rationally develop optimal strategies for maximizing the anti-tumor activity of γδ T cells and inhibiting their pro-tumor activity. Here, we summarize the recent progress regarding the immunobiology of human γδ 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 γδ T cells.

ORIGIN AND DEVELOPMENT OF THE HUMAN γδ T CELLS

The γδ T cells develop earlier than αβ 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 γδ T cells at the embryonic day of 14, Vγ5+Vδ1+ 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 Vγ6'Vδ1' T cells that are destined for the female genital tract, peritoneal cavity and tongue, and other γδ T cells with diverse VDJ clonotypes containing Vγ1, 2, 4, and 7 segments (18,22). Whereas the early developing γδ T cells have invariant TCRs, the γδ T cells appearing during the later period of the development are diverse in TCR repertoire (22-24).

Human γδ T cells are also present in the thymus as well as the periphery, suggesting the thymic development of human γδ T cells (25). Although adult blood γδ T cells are predominated by Vγ9Vδ2 cells, neonatal cord blood γδ T cells express a diversity of Vγ and Vδ chains paired in various combinations, and the majority of neonatal γδ T cells are Vγ9Vδ1' cells (26,27). Therefore, the adult blood Vγ9Vδ2 cells appear to represent the post-natal expansion of Vγ9Vδ2 cells expressing canonical CDR3s in response to microbial phosphoantigens that are described below (28-30). Human Vγ9Vδ2 cells have been shown to expand rapidly after birth within 1 year of life (31). In the adult, Vδ1 and Vδ2 γδ T cells are localized in the barrier tissues and the peripheral blood, respectively (32).

HETEROGENEITY OF THE HUMAN γδ T CELLS

Although γδ T cells are cousins of αβ T cells, γδ T cells directly recognize Ags via their γδ TCRs without the need of MHC molecules similarly to B cells (1,33,34). The γδ 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 γδ T cells exhibit highly adaptive features such as clonal expansion and differentiation from naïve cells to effector cells (35). The overall characteristics of γδ T cells may be positioned between NK cells and CD8+ T cells (36). The γδ T cells are heterogeneous concerning functional features depending on the usage of TCR γ and δ chains and the tissue localization. The γδ T cell population consists of tissue-resident and peripheral blood γδ T cells (1). Human γδ T cells account for 0.5%-5% of all peripheral blood T cells (2,7).
In humans, several functional Vγ gene segments (including Vγ2, Vγ3, Vγ4, Vγ5, Vγ8, Vγ9, and Vγ11) rearrange into 5 Jγ segments and 2 Cγ segments on chromosome 7 to generate TCRγ chains, whereas TCRδ chains are generated by the rearrangement of at least 7 Vδ, 3 Dδ, 3 δ, and 1 Cδ segments on chromosome 14 (2,3,7,37). Whereas Vδ1, Vδ2, and Vδ3 segments are used only in the rearrangement of the TCR δ chains, Vδ4–Vδ7 segments are also used in the rearrangement of the TCR α chains and have alternative gene names belonging to TCR Vα gene segments (37). The functional features of γδ T cells are closely correlated with the usage of the TCRδ chains (24). Among 7 Vδ segments, Vδ1 and Vδ2 segment-using γδ TCRs are the most common human γδ TCRs (2).

As the most abundant human γδ T cells are Vγ9Vδ2 T cells that recognize unique phosphoantigens and Vδ1 T cells have adaptive features distinct from Vγ9Vδ2 T cells, γδ T cells are commonly classified into Vδ2 and non-Vδ2 γδ T cells (2,35,38). The Vγ9Vδ2 T cells are the most well-known human γδ T cells and have been exploited for anti-cancer immunotherapy (10,11). The characteristics and the adaptive features of non-Vδ2 γδ T cells, especially Vδ1 γδ T cells, are recently recognized, and these Vδ1 γδ T cells are also thought to be a candidate for anti-cancer immunotherapy (35).

THE γδ TCR STRUCTURE AND ACTIVATION OF THE HUMAN γδ T CELLS

Although γδ T cells share TCR rearrangement mechanism and memory functions with αβ T cells, they differ in the immune response kinetics and mechanisms of target cell recognition (39). The γδ T cells do not recognize MHC molecules, but many γδ 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 αβ TCRs, γδ TCRs are also associated with CD3 molecules, but differently from murine αβ TCRs, murine γδ TCRs contain only CD3γδ dimers, not CD3γε dimers (40). Notably, murine γδ TCR cells can develop in the absence of CD3ε or CD3δ (41,42), but the expression of CD3γ is indispensable for the murine γδ T cell development (43). Furthermore, CD3ζ chain is not necessary for the γδ T cell development and FcγRIγ chain, a CD3ζ chain family member that can dimerize with CD3ζ, is expressed upon activation and then included in the γδ TCR complexes (44). On the other hand, human γδ TCR complex contains CD3δ chain and shows a TCRγδCD3ε,δζ2 stoichiometry similarly to human αβ TCR complex, whereas mouse γδ TCR complex has a TCRγδCD3εγζ2 stoichiometry (45). Human γδ TCR signaling is less dependent on CD3γ chain than CD3δ chain as human patient lacking CD3γ have abundant peripheral blood γδ T cells expressing high levels of γδ TCR (46). Interestingly, forced expression of human, but not murine, CD3δ transgene rescue the γδ T cell development in mice deficient in both CD3δ and CD3γ genes, suggesting the unique role of human CD3δ in the TCR signaling (45).

The γδ TCR signaling is qualitatively different from the αβ TCR signaling (44). The γδ TCRs self-oligomerize and cause constitutive signaling in the absence of ligands (47). These γδ TCR signaling characteristics are similar to those of pre-αβ TCR signaling responsible for the β selection during thymic T cell development (48). During the thymic γδ T cell development, γδ T cells that encounter strong agonistic ligands obtain the capability of secreting IFN-γ. In contrast, γδ T cells that do not encounter strong agonists adopt IL-17-default position (1,47).
In the periphery, the stimulation of γδ T cells via γδ TCR and costimulatory receptors or NK cell receptors triggers γδ 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, γδ T cells can also exert a potent cytotoxic activity without the obligatory delay associated with clonal expansion and differentiation (49).

Although γδ TCR is regarded as an activating receptor, γδ TCR may act as an inhibitory receptor in certain contexts. The consequence of the constitutive γδ TCR signaling can be inhibition of γδ 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 Vγ5δ1 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 γδ T cells have a lot of NK cell-activating receptors such as NCRs and NKG2D (8,9), the functional roles of γδ TCR should be carefully investigated in heterogeneous subpopulations of γδ T cells since the NK receptors, not γδ TCR, could be main receptors for γδ T cell activation. The NK cell-activating receptors can be considered as costimulatory receptors if γδ TCR and NK cell receptors induce synergistic signaling for γδ T cell responses (52). The list of costimulatory receptors for αβ T cells has been expanded and includes a prototype costimulatory molecule CD28 (53). The relevance of costimulatory molecules for αβ T cells in γδ T cells remains debatable. About 40%–60% of γδ T cells express CD28, and the expression of CD28 is decreased upon the activation of γδ T cells (54,55). Since anti-CD28 agonistic Abs enhance human γδ T cell proliferation, the role of CD28 as a costimulatory molecule is valid in a subpopulation of human γδ T cells. Considering the phenotypes of memory and effector CD8+ αβ T cells (56), it may be hypothesized that the expression of CD28 is lost upon the prolonged activation of a subpopulation of γδ T cells. It is noteworthy that a higher proportion of Vδ1 γδ T cells do not express CD28 than that of Vδ2 γδ T cells, but the most of Vδ2 γδ T cells express CD28 similarly to naïve αβ T cells (35,57).

**THE γδ T CELLS IN THE TUMOR MICROENVIRONMENT (TME)**

**Recruitment of human γδ 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 γδ 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-
anti-inflammatory cytokines and their interactions with different types of innate and adaptive immune cells in the TME (7,49,63,64). The γδ 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-1α and MIP-1β (11,35,65).

**Major tumor-infiltrating γδ T cell subsets: human Vδ1 and Vγ9Vδ2 T cells**

In humans, Vδ1 and Vγ9Vδ2 T cells are 2 main populations of γδ 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 Vδ1 and Vγ9Vδ2 T cells have the cytotoxic capability and can have anti-cancer activity (11,36). The 2 subsets of γδ 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 Vγ9Vδ2 T cells are the most predominant γδ T cells in the adult peripheral blood, but they are not a major γδ T cell population at the time of birth as the Vδ1 γδ T cells are predominant during fetal and early life (24,31). The Vγ9Vδ2 T cells expand postnatally in response to phoshoantigens by microbes. The canonical Vγ9Vδ2 T cells with Vγ9JγP sequences recognize phosphoantigens presented by butyrophilin 3A (BTN3A). Interestingly, prenyl pyrophosphates (phosphoantigens) bind to the intracellular B30.2 domain of BTN3A1,
Heterogeneity of Human γδ T Cells and Cancer Immunity

In general, the extent of intratumoral DCs, NK cells, neutrophils, T cells and B cells in the TME (1,4,22,75,77,82,85). exhibit indirect anti-tumor responses by modulating different immune cell types including cellular cytotoxicity using cell surface CD16 (FcγRIII) similar to NK cells (86). Lastly, γδ T cells exhibit indirect anti-tumor responses by modulating different immune cell types including DCs, NK cells, neutrophils, γδ T cells and B cells in the TME (1,4,22,75,77,82,85).

The γδ T cells have both anti-tumor and pro-tumor activities

Upon migration to the TME, γδ T cells exert potent anti-tumor effects via multiple mechanisms (77,82). The γδ 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 γδ T cells are also able to kill Ab-coated cancer cells by Ab-dependent cellular cytotoxicity using cell surface CD16 (FcγRIII) similar to NK cells (86). Lastly, γδ T cells exhibit indirect anti-tumor responses by modulating different immune cell types including DCs, NK cells, neutrophils, γδ T cells and B cells in the TME (1,4,22,75,77,82,85).

In general, the extent of intratumoral γδ T cell infiltration is highly associated with the CD8+ T cell signature and patients’ prognosis, suggesting that γδ T cells largely perform anti-tumor
activity rather than pro-tumor activity (87, 88). However, complex interactions between TME and intratumoral γδ T cells can result in the diversion of anti-tumor γδ T cells into pro-tumor cells. Therefore, the precise role of γδ T cells in each individual patient may depend on the specific γδ 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 γδ 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 γδ T cells can also be classified as γδ Th1, γδ Th2, γδ Th17, γδ Tfh, and γδ Treg cells based on their functional polarization (1, 7, 22, 35, 49, 75, 77, 85, 92). Interestingly, in response to different cytokines, γδ T cells can trans-differentiate from one phenotype to another (1, 7, 22, 35, 49, 75, 77, 85, 92). Both Vδ1 and Vγ9Vδ2 T cells can be polarized into γδ Th1 cells, γδ Thfh cells, γδ T17 cells, γδ Treg cells, and γδ Th2 cells with distinct cytokines. It is important to investigate further whether γδ T cells are a primary driver of T cell polarization or whether the immunotherapy targeting γδ T cells can change the overall polarization of αβ T cells within the TME.

The γδ T cells are also subjected to immune exhaustion similarly to cancer-reactive cytotoxic T cells and NK cells. Although the nature of the γδ T cell immune exhaustion is not well reported, prolonged stimulation of γδ 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 γδ T cells are exhausted and whether exhausted γδ T cells can be easily reawakened by strong stimuli, including cytokines or Ags such as phosphoantigens.

**FUTURE DIRECTIONS FOR OPTIMIZING ADOPTIVE γδ T CELL TRANSFER AS AN ALTERNATIVE CANCER IMMUNOTHERAPY**

The ability of γδ 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, γδ T cells also can be considered as universal allogeneic adoptive T cell transfer for cancer patients. Accordingly, recent applications of γδ 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 γδ T cells. Furthermore, the anti-tumor function of γδ 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 γδ T cell immunotherapy lie in the quest to minimize activation-induced γδ T cell death, anergy, and the polarization to specific γδ 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 γδ 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 γδ T cells. For example, IL-36γ acts synergistically with TCR signaling and is able to promote IFN-γ production by CD8+ T cells, NK cells, and γδ 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 Vγ9Vδ2 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 Vδ1 T cells compared with that of Vγ9Vδ2 T cells, at least in the context of certain tumors (14,67,77,98,99,106-110), 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. An interesting property of Vδ1 T cells for the adoptive transfer approach is their CCL2-mediated chemotaxis toward tumors (67,111,112). Vδ1 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 Vδ1 T cells and simultaneously inhibits Vδ2 T-cell growth (77,80,113), thus providing a novel basis to develop the preferential expansion approaches for Vδ1 T cells.

CONCLUDING REMARKS

Although γδ 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 γδ T cells for cancer immunotherapy, it is critical to better characterize human γδ 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 γδ T cells so that γδ T cells can be durably activated with a potent anti-tumor phenotype. To maintain the anti-tumor activity of γδ T cells for a long period of time, the specific depletion of pro-tumor γδ T cells before the immunotherapy, the co-transfer of other immune cells that activate γδ T cells, and the modification of the cytokine balance in the TME should be considered in the immunotherapy using γδ T cells. In summary, as γδ T cells are heterogeneous, the pro-tumor or anti-tumor activities of different γδ T cell populations need to be thoroughly delineated and utilized to maximize the efficacy of the immunotherapy using γδ T cells.

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