Molecular Determinants of Target Cell Recognition by Human γδ T Cells

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The unique capabilities of gamma-delta (γδ) T cells to recognize cells under stressed conditions, particularly infected or transformed cells, and killing them or regulating the immune response against them, paved the way to the development of promising therapeutic strategies for cancer and infectious diseases. From a mechanistic standpoint, numerous studies have unveiled a remarkable flexibility of γδ T cells in employing their T cell receptor and/or NK cell receptors for target cell recognition, even if the relevant ligands often remain uncertain. Here, we review the accumulated knowledge on the diverse mechanisms of target cell recognition by γδ T cells, focusing on human γδ T cells, to provide an integrated perspective of their therapeutic potential in cancer and infectious diseases.

Keywords: gamma-delta T cell, T cell receptor, NK cell receptor, NKG2D, tumor immunology

INTRODUCTION

More than three decades after the discovery of gamma-delta (γδ) T cells (1), the research community is still missing a compelling picture about their mechanisms of activation and target cell recognition. Despite the relatively small abundance of γδ T cells in the human blood, it is clear that this lymphocyte population plays an important role at the interface between the innate and the adaptive immune systems. These cells share T cell receptor (TCR) rearrangements and memory functions (2) with their αβ T cell counterparts, but differ in their response kinetics and mechanisms of target cell recognition. Thus, γδ T cell activation is typically independent of antigen presentation by major histocompatibility complex (MHC) molecules. Furthermore, γδ T cells bear a plethora of NK cell receptors (NKRs) on their surface, which allow for very fast responses against infected or transformed cells (3), thus contributing to a first line of defense that precedes antigen-specific αβ T-cell responses (4).

Unlike αβ T cells, there is little evidence of thymic negative selection of self-reactive γδ T cells. Vγ9Vδ2 T cells, which constitute the major (60–95%) γδ T cell subtype in humans, seemingly expand in the periphery in response to microbial or stress-induced phosphorylated antigens (2) while displaying preferential Vγ9-JP TCR rearrangements (5). Other human γδ T cell subsets, namely Vδ1+ and Vδ3+ T cells that are highly reactive to cytomegalovirus (CMV) infection (6), display TCR repertoires biased toward sequences recognizing CMV-infected cells (7). But while Vγ9Vδ2 TCR recognition has been well characterized and discussed (5, 8), it remains less clear how other γδ T cell subsets are activated to participate in lymphoid stress surveillance (9).

The purpose of this review is to discuss the current knowledge on target cell recognition by human γδ T cells (Table 1), emphasizing the role of the TCR as well as NKR ligands, in the context of cancer and infectious diseases.

TUMOR CELL RECOGNITION

Early research on the molecular mechanisms of γδ T cell recognition in the 1990s led to the realization of its unusual independence of peptide processing and MHC-restricted presentation, in marked contrast with αβ T lymphocytes (42–44). One of the first lines of evidence came from non-peptidic
TABLE 1 | Tumor- or infected cell-associated ligands recognized by gamma-delta (γδ) T cells.

| Ligand | Receptor | γδ subset | Infection/cancer | Reference |
|--------|----------|-----------|-----------------|-----------|
| CD1 proteins + endogenous or exogenous lipids | T cell receptor (TCR) | Vγ9Vδ2 | Infection | (10, 11) |
| BTN3A1 + phosphoantigens | TCR | Vγ9Vδ2 | Infection | (9) |
| Endothelial protein C receptor | TCR | Vγ4Vδ5 | Both | (12) |
| Annexin A2 | TCR | Vγ8Vδ3 | Both | (13) |
| Heat shock protein 60 | TCR | Both | (14–17) |
| F1-ATPase | TCR | Cancer | (18) |
| SEA and SEE | TCR | Infection | (19) |
| OXYS | TCR | Infection | (20) |
| DXS2 | TCR | Infection | (21) |
| Glycoprotein I | TCR | Infection | (21) |
| MSH2 | TCR | Both | (14, 22) |
| NKG2D | | | |
| HLA-E | NKG2C | Infection | (23) |
| HA | Sialic acid receptor | Infection | (24) |
| CD48 | 2B4 | Cancer | (25–27) |
| MICA/MICB | TCR | Vδ1 | Both | (28–30) |
| MICA | NKG2D | Vγ9Vδ2 | Cancer | (33) |
| UL16 binding protein (ULBP)1 | NKG2D | Vγ9Vδ2 | Cancer | (34) |
| ULBP2 | NKG2D | Vδ1 | Cancer | (35, 36) |
| ULBP3 | NKG2D | Vδ1 | Cancer | (35–37) |
| ULBP4 | TCR and NKG2D | Vδ2 | Cancer | (38) |
| ? | Nkp30 | Vδ1 | Both | (39, 40) |
| PVR/Nectin-2 | DNAx accessory molecule 1 | Vγ9Vδ2 | Cancer | (41) |

*“?” means undescribed/unknown in the referenced studies.

prenyl pyrophosphates (“phosphoantigens” (PAg)) recognized by Vγ9Vδ2 TCRs (45, 46). Initially, bacteria and parasites were shown to produce strong PAg agonists for Vγ9Vδ2 TCRs (47), but later it became clear that these could also be activated by weaker agonists, such as isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate, that are natural intermediates of the mevalonate pathway of isoprenoid and steroid synthesis in eukaryotic cells (48). Importantly, the dysregulation of the mevalonate pathway in some tumor cells allows for the accumulation of these (weaker) PAgs, thus promoting Vγ9Vδ2 TCR-mediated recognition (49). Furthermore, treatment with zoledrone or pamidronate (which are approved drugs) was shown to be very effective at inducing the accumulation of intracellular PAgs like IPP, and thus potentiate TCR-dependent Vγ9Vδ2 T cell cytotoxicity against tumor cell targets, including cancer stem cells (50).

A key recent breakthrough was the discovery of butyrophilin-related proteins, especially BTN3A1, as major molecular determinants of Vγ9Vδ2 TCR-mediated recognition of PAgs, even if the underlying mechanism has gathered some controversy. A model supporting extracellular PAg presentation to the Vγ9Vδ2 T cell (in a MHC-like manner) was first proposed, with biophysical and structural data in support (51). However, following reports demonstrated that PAgs interact directly with the intracellular B30.2 domain of BTN3A1 through a positively charged surface pocket; and that charge reversal of pocket residues abrogates PAg binding and Vγ9Vδ2 T cell activation, with no detectable association with the extracellular domain of BTN3A1 (13, 52, 53). More recently, it has been shown that changes in the juxtamembrane domain of BTN3A1, which is located close to the start of the B30.2 domain, induced marked alterations in Vγ9Vδ2 T cell reactivity, thus highlighting the importance of the intracellular domain for the correct Vγ9Vδ2 T cell function and activation (54). Because of its location between the intracellular and the extracellular domains, the B30.2 domain seems critical in translating the pAg-induced conformational change of BTN3A1 from the inside to the outside of the target cells (55, 56).

Besides sensing PAgs, γδ T cells seemingly recognize transformed cells through proteins that are expressed at the cell surface in a stress-induced manner. Some examples are typically endogenous proteins, such heat shock protein 60 (14–17) or FI-ATPase (18), that can be ectopically expressed on the cell membrane upon transformation and recognized by Vγ9Vδ2 TCRs to promote tumor cell lysis. More recently, endothelial protein C receptor (EPCR), which acts on the coagulation cascade, was shown to be expressed on the cell surface during transformation and recognized by a non-Vδ2 (Vγ4Vδ5) TCR (12). Similarly, Annexin A2, expressed on tumor cells in response to increasing quantities of reactive oxygen species, engaged directly with a Vγ8Vδ3 TCR (13). The identification of these rather different ligands highlights the complexity of tumor cell recognition via γδ TCRs. This notwithstanding, it is clear that γδ T cells also rely on “NK-like” mechanisms for tumor cell recognition, using receptors such as 2B4 and NKG2D, originally thought to be specific to NK cells.

The first indication of an NK-like recognition mechanism was unveiled upon the ability of stimulated murine γδ T cells to recognize CD48 (25, 26), a well-known 2B4 ligand, suggested to work as an accessory molecule that strengthens effector–target interactions (27). Surprisingly, only the 2B4+ γδ T cells were able to develop non-MHC-restricted cytotoxicity against lymphoma cells (57, 58). Although 2B4 is also expressed on activated human γδ T cells, its relevance is still unclear as 2B4 engagement failed to promote proliferation or cytokine production (59).

Much more consensual is the role of NKG2D, which is widely expressed not only in NK cells but also in most γδ and some αβ
In human γδ T cells, both Vδ1+ and Vδ2+ subsets, NKG2D was shown to be responsible for recognition of tumor cells expressing MHC class I chain-related (MIC) A/B (28, 29, 31–33, 62) or UL16 binding protein (ULBP) 1/2/3/4 (34–38, 50, 63) ligands. In fact, high tumor carcinoma samples from lung, breast, kidney, ovary, and prostate cancers expressing MIC or MICB presented higher of infiltrating V61+ T cells, which in turn were able to target and kill autologous and heterologous tumor cells (25, 59). Our group’s work revealed that ULBP1 was particularly important for leukemia and lymphoma cell recognition by PAg-activated Vγ9Vδ2 T cells (34). Notwithstanding, one should note the relevance of a synergistic TCR engagement for an efficient cytotoxic response (37, 38). In fact, some works suggested that MIC or ULBP recognition by γδ T cells is not only restricted to NKG2D but also involves the γδ TCR (26, 31). A similar recognition pattern was also observed against human MutS homolog 2 (hMSH2) ectopically expressed in epithelial tumor cell lines. Both TCRγδ and NKG2D were able to interact with hMSH2 and contribute to Vδ2+ γδ T cell-mediated cytotoxicity and interferon γ (IFN-γ) production (14, 22).

Besides 2B4 and NKG2D, DNAX accessory molecule 1 (DNAM-1) was also shown to be widely expressed in V81+, Vδ2+, and Vδ1-Vδ2 γδ T cell subsets (64); and masking DNAM-1 on γδ T cells significantly inhibited tumor cell killing (64, 65). DNAM-1-dependent γδ T cell recognition was reported for hepatocellular carcinoma (41), acute (65) and chronic (64) myeloid leukemia, and multiple myeloma (66) cell lines. More specifically, Vγ9Vδ2 T cells were shown to use DNAM-1 to interact with Nectin-2 and PVR that are widely expressed in the tumor cell targets (41, 65). Curiously, PVR engagement potentiated γδ T cell cytotoxicity, whereas Nectin-2 blocking did not affect it (41). Tumor targets that expressed both DNAM-1 and NKG2D ligands were able to engage both receptors on γδ T cells, having a synergistic effect on their cytolytic activity (41, 64, 66). Moreover, therapeutic strategies that enhanced the expression of NKG2D or DNAM-1 ligands, such as MICA/B and ULBP1/2, or Nectin-2 and PVR, respectively, potentiated γδ T cell recognition of colon cancer, glioblastoma, multiple myeloma, and lymphoma cells (67–70).

From a therapeutic perspective, γδ T cell recognition of tumor cells may also rely on the induced expression of natural cytotoxicity receptors (NCRs) that recognize a distinct set of tumor-associated ligands, such as B7-H6 or BAT3 (71). Thus, our group has shown that NKP30 and NKP44 can be reproducibly induced in vitro in V61+ (but not Vδ2+) γδ T cells (39). A very mild expression of NKP44 on expanded γδ T cells had been reported before (72); and shown to contribute γδ T cell cytotoxicity against myeloma cells (61). In our studies, we observed not only a robust expression of NKP44 but also NKP30, in V61+ γδ T cells activated with TCR agonists and IL-15 (or IL-2); and both receptors enhanced γδ T cytotoxicity against tumor target cells (39, 73). Among the various known ligands for NCRs, it is still unclear which are most relevant for NCR+ V61+ γδ T cell recognition of tumor cells. While the NKP30 ligand, B7-H6, is an obvious candidate (74), a very recent report identified an unanticipated ligand for NKP44 in the form of platelet-derived growth factor (PDGF)-DD (75), known for its capacity to promote of tumor cell proliferation, epithelial-mesenchymal transition, and angiogenesis. PDGF-DD ligation to NKP44 enhanced IFN-γ and TNF-α secretion (by NK cells), which in turn induced tumor cell growth arrest (75). Additional investigation will be needed to elucidate the relative importance of NCR, NKG2D, DNAM-1, or TCR ligands in tumor cell recognition by γδ T cells, aiming to maximize their potential in cancer immunotherapy.

**INFECTED CELL RECOGNITION**

Multiple lines of evidence since the late 1980s have shown that γδ T cells display strong activities against bacteria, including *Mycobacterium tuberculosis* (76–81); parasites, such as *Plasmodium falciparum* (82–86); and viruses (87, 88), most notably CMV (89–91). Vγ9Vδ2 T cells can be specifically and potently activated by PAgS like (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate, an intermediate of the 2-C-methyl-d-erythritol 4-phosphate pathway employed by eubacteria and apicomplexan protozoa but not by eukaryotes (48, 92, 93). This likely underlies the striking expansions of Vγ9Vδ2 T cells in individuals infected with *M. tuberculosis* (76–81) or *P. falciparum* (83). Besides PAgS, several other molecules of microbial origin have been proposed as γδ T cell antigens accounting for the specific recognition of infected cells. These candidates include the bacterial superantigens SEA (and to a lesser extent SEE) (19); OXYS and DXS2, two mycobacterial proteins found to activate γδ T cells from BCG-infected human subjects but not from healthy donors (20, 21); and HSV-1 glycoprotein I, specifically recognized by a Vγ1.2Vδ8 TCR independently from antigen processing and MHC presentation (20, 21).

Subsequent reports demonstrated that γδ T cells also recognize stress antigens of cellular origin, either in antibody-like or antigen-presentation-like fashion. γδ T cells can indeed directly recognize stress proteins like hMSH2, a nuclear protein ectopically expressed on the cell surface of different epithelial tumor cells and induced by EBV transformation (22); and Annexin A2 whose expression was induced by CMV infection and recognized specifically by a Vγ8Vδ3 T cell clone (13). On the other hand, γδ T cells can recognize nonpolymorphic MHC-like (class Ib) proteins presenting lipids, such as CD1 proteins, in a similar way to other unconventional T cells like NKT or MAIT cells (11, 94–96). In particular, a subpopulation of V81+ T cells has been clearly shown to bind CD1d loaded with the self-lipid sulfatide (97) but any concrete link to the recognition of infected (or transformed) cells remains to be established. Of note, another CD1-like protein, EPCR, was shown to bind directly (independently of lipid cargo) the TCR of a Vγ4Vδ5 T cell clone (expanded from a CMV+ individual), thus allowing it to recognize endothelial cells infected with CMV (12).

In addition to the TCR, γδ T cells can also use NKG2D to recognize infected cells infected with various viruses and intracellular bacteria (32, 98–102). More specifically, the stress-inducible molecule, MICA, was induced on the surface of dendritic and epithelial cells by *M. tuberculosis* infection in *vivo* and in *vitro*; and its binding to NKG2D, substantially enhanced the TCR-dependent Vγ9Vδ2 T cell response to PAgS (28). Furthermore, in the case of *Brucella*, ULBP1 was the main NKG2D ligand upregulated on infected macrophages, and its engagement promoted Vγ9Vδ2 T cell cytotoxicity and cytokine production, which contributed to the inhibition of bacterium development (100).
A few other receptors have implicated in γδ T cell recognition of infected cells. Thus, another NKR, NKG2C, constitutively expressed on V61+ T cells, induced a cytolytic response against HIV-infected CD4+ T cells expressing its ligand, HLA-E (23). On the other hand, we found that NKp30 can also play an important role in HIV-1 infection upon its induced expression in Vδ1+ T cells; NKp30 ligation triggered the production of CCL3, CCL4, and CCL5 chemokines that suppressed the replication of a CCR5-tropic strain of HIV-1 (40). Finally, in the case of avian influenza (H5N1), γδ T cells were reported to use sialic acid receptors for infection of infected or transformed cells by the virus (24). To understand how different microorganisms may elicit distinct pathways of γδ T cell recognition of pathogen-associated or stress-induced antigens remains a challenge for future research.

CONCLUDING REMARKS

In contrast with the well-established paradigm of MHC-restricted recognition of peptides by conventional αβ T cells, or even MHC class Ib-dependent recognition of lipids by unconventional αβ T cells, the molecular mechanisms of target cell recognition by γδ T cells remain poorly understood. A notable exception is the BTN3A1-mediated sensing of PAgS by γ9Vδ2 T cells, which underlies their responses to tumors and infections like TB or malaria. For most other γδ T cell subsets, however, TCR specificities are either unknown, not generalizable or of unclear physiological relevance. Therefore, the identification of relevant, non-Vγ9Vδ2 TCR ligands remains a major challenge in the γδ T cell field.

On the other hand, while NKRs are also clearly involved in γδ T cell recognition of tumor or infected cells, we still lack appropriate understanding how the multiple signals derived from all the expressed NKRs are integrated, also with those coming from the TCR itself. This likely depends on the relative expression levels of the various putative NKR and TCR ligands in each target cell, which adds significant complexity to the process of γδ T cell recognition.

The broad spectrum of MHC-unrestricted recognition of infected or transformed cells by γδ T makes them attractive candidates for adoptive cell therapy (ACT). All clinical trials have thus far concentrated on γ9Vδ2 T cells, probably due to their relative abundance in the peripheral blood and especially the availability of FDA-approved drugs, such as zoledronate and pamidronate, that allow their activation and expansion in vivo (103). γ9Vδ2 ACT has shown promising pre-clinical results against TB (104) and has already been tested in various cancer clinical trials [reviewed in Ref. (105)] that documented its safety and some (albeit still sub-optimal) efficacy (106–108). This could be maybe explained by γ9Vδ2 T cell susceptibility to exhaustion and activation-induced cell death (AICD). Nonetheless, improvements in γ9Vδ2 ACT protocols may still increase their efficacy, as indicated by some studies with exogenous provision of IL-2, importantly without the need for lymphodepleting preconditioning (109, 110). As for Vδ1+ γδ T cells, they are less susceptible to AICD and exhaustion when compared to γ9Vδ2 T cells (111). However, no clinical trial has yet focused on this γδ T cell subset, mostly due to the lack of clinical-grade protocols allowing their successful expansion. Importantly, we have recently developed a clinical-grade process to effectively expand Vδι+ T cells while also inducing NCR (and augmenting NKG2D) expression; and established the proof-of-concept in leukemia xenograft models (73). We further anticipate NCR+ Vδ1+ ACT to be a promising therapeutic strategy also for solid tumors and chronic viral infections.

AUTHOR CONTRIBUTIONS

AS, BL, and BS-S conceived and wrote the manuscript. AS and BL contributed equally to the manuscript.

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Conflict of Interest Statement: BS-S is a co-founder and shareholder of Lympahc—Lymphocyte Activation Technologies S.A. The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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