Review

γδ T-cells: basic features and potential role in vasculitis

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ABSTRACT

γδ T-cells are a numerically small subset of T-cells with distinct features. They recognise antigens that are not seen by other immune cells. At the functional level, γδ T-cells share some features with αβ T-cells but also exert functions that are otherwise performed by specialised subsets of αβ T-cells (e.g. IL-17 production, regulatory activity). We discuss the potential role of γδ T-cells in various clinical forms of vasculitis.

Introduction

Most peripheral T-lymphocytes express a disulphide-bridged αβ T-cell receptor (TCR) heterodimer. αβ T-cells recognise short peptides presented on MHC class I molecules (to CD8 T-cells) or MHC class II molecules (to CD4 T-cells). Due to the large number of available variable (V) and joining (J) gene segments in the TCR α and β loci, the expressed TCR repertoire of αβ T-cells is very diverse. A second T-cell subset expresses the alternative γδ TCR, which is composed of a γ and δ chain heterodimer. In striking contrast to αβ T-cells, the number of available Vγ and Vδ genes is small. γδ T-cells account for approximately 5% of T-cells in the peripheral blood but constitute a major population in other anatomical localisations such as the small intestine and some epithelia (1,2). Two reasons might be envisioned why nature has afforded to maintain two separate sets of T-cells throughout evolution. First, the two T-cell subsets might be endowed with distinct functional capacities. Secondly, the TCR of the two subsets might be destined to recognise separate ranges of antigenic moieties. Accumulated evidence would speak in favour of the latter alternative. Over the years, many studies have analysed the functional activity of murine and human γδ T-cells. Depending on the cellular context and the activation signals, γδ T-cells can produce a range of cytokines including interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and many chemokines (1,3-6). γδ T-cells freshly isolated from human peripheral blood tend to be primed toward a Th1 phenotype but can be induced under appropriate *in vitro* culture conditions to produce the Th2 cytokine IL-4 (7). Strikingly, γδ T-cells are poor IL-2 producers, which explains the T helper cell-dependency of γδ T-cell activation *in vitro* (8). γδ T-cells localised in specialised areas such as the mucosal tissues, epithelia and epidermis can produce additional cytokines with relevance for the local immune response. Thus, intraepithelial and epidermal γδ T-cells contribute to epithelial cell growth and wound repair through the production of keratinocyte growth factor/fibroblast growth factor-7 and insulin-like growth factor-1 (9-11). Recently, γδ T-cells have been recognised as an important cellular source of the pro-inflammatory cytokine IL-17 (see below). In addition to the production of soluble mediators, γδ T-cells usually display potent cytotoxic effector activity. γδ T-cells can kill susceptible target cells by the secretory pathway through the production of perforin and granzymes, but can also make use of the receptor-ligand (*e.g.* Fas/Fas-ligand) pathway (12-14). Taken together, it appears that the effector functions of γδ T-cells are not fundamentally different from αβ T-cells, with the possible exception of the production of particular mediators in selected areas of the body.

Ligands recognised by γδ T-cells

It is by now well documented that the TCR of a particular subset of human γδ T-cells recognises antigens that are not seen by any other immune receptor.

The few expressed Vγ and Vδ genes are not randomly used by human γδ T-cells. Instead, there is a clear preponderance of cells expressing one particular VγVδ combination, *i.e.* Vγ9 and Vδ2, among circulating γδ T-cells in the human blood. Vγ9Vδ2 T-cells account for 50 to >90% of peripheral blood γδ T-cells in most healthy adult individuals (15, 16). It has been shown that the Vγ9Vδ2 TCR recognises non-peptidic phosphorylated molecules that are intermediates of the isoprenoid biosynthesis pathway required for the prenylation of proteins and the synthesis of cholesterol (17). There are two separate pathways leading to the synthesis of the isoprenoid metabolite isopentenyl pyrophosphate (IPP): While eukaryotic cells use the mevalonate-dependent pathway, most prokaryotic cells synthesise IPP *via* the non-mevalonate (or "Rohmer") pathway (18). IPP is recognised by the Vγ9Vδ2 TCR but high concentrations (in the micromolar range) are required; while non-transformed normal cells produce too little IPP to activate γδ T-cells, transformed tumour cells produce much higher amounts of IPP, which can then be sensed by Vγ9Vδ2 T-cells, rendering many tumour cells susceptible to γδ T-cell-mediated lysis (19). Interestingly, the intracellular levels of IPP production in eukaryotic cells can be easily modulated by clinically used drugs, *i.e.* aminobisphosphonates. Aminobisphosphonates inhibit the IPP-degrading enzyme farnesyl diphosphate synthase, resulting in the intracellular accumulation of IPP (19, 20). As a consequence, treatment with aminobisphosphonates or siRNA-mediated inhibition of farnesyl diphosphate synthase increases the susceptibility of tumour cells to γδ T-cell-dependent lysis (21). In contrast to eukaryotic cells, bacteria and some parasites use the non-mevalonate pathway for the synthesis of IPP. Interestingly, intermediates of this pathway upstream of IPP have been found to be the most potent TCR-dependent activators of human Vγ9Vδ2 T-cells. For example, only pico- to nanomolar concentrations of (E)-1-hydroxy-2-methylbut-2-enyl 4-diphosphate (HMB-PP) are required to stimulate human Vγ9Vδ2 T-cells (22, 23). The recognition of a unique class of pyrophosphate ligands that are secreted by bacteria or are overproduced by (some) malignant cells assigns an important function in immune surveillance to the human Vγ9Vδ2 T-cell subset. The very same T-cell population thus plays an important role in immune defense against infection and in the elimination of transformed cells through the recognition of related molecules (17, 24, 25). In contrast to the peptide presentation to $CD4^+$ and $CD8^+$ αβ T-cells, the presentation of such phosphoantigens is MHC-independent. As a consequence, the activation of Vγ9Vδ2 T-cells is not MHC-restricted (1, 26). Moreover, all T-cells displaying the Vγ9Vδ2 TCR (*i.e.* on average 2-5% of all peripheral blood T-cells) are activated by phosphoantigens, independently of CDR3 sequence variability. Thus, Vγ9Vδ2 T-cells use their TCR as a pattern recognition receptor, comparable to well characterised pattern recognition receptors such as the Toll-like receptor family (25). This feature, together with the rapid and sustained activation of effector functions (27, 28), places γδ T-cells as a potential link between the innate and adaptive immune system (29).

In view of the exclusive features of human Vγ9Vδ2 γδ T-cells, it is surprising to note that there is no homologous TCR in the murine γδ TCR repertoire that would enable mouse γδ T-cells to recognise microbial or eukaryotic phosphoantigens. However, the recognition of stress-induced ligands has emerged as a common theme of both murine and human γδ T-cells. MHC class I-related molecules that are up-regulated upon cellular stress (*e.g.* heat-shock, DNA damage, infection), or are constitutively expressed on some normal and/or on transformed cells, have been shown to be recognised by specified murine or human γδ TCRs. For instance, murine non-classical MHC class I molecules with constitutive (T22) or inducible expression (T10) have been identified as ligands for the murine G8 γδ T-cell hybridoma, but some mouse γδ T-cells can also recognise peptide-MHC class II I-E complexes (30). In humans, γδ T-cells localised in mucosal tissue and expressing the TCR Vδ1 chain (in contrast to the above mentioned blood Vγ9Vδ2 T-cells), have been described to recognise stress-inducible MHC class I-related chain A (MICA) antigens that are inducibly expressed on epithelial cells and frequently constitutively expressed on tumour cells (31). Taken together, it seems reasonable to assume that γδ T-cells use their TCR to control the integrity of tissue and to sense danger signals as generated by infection, inflammation, and cellular transformation.

Like conventional αβ T-cells, γδ T-cells express additional cell surface molecules with regulatory function on T-cell activation. An important activating receptor is the natural killer cell group 2, member D (NKG2D) receptor, a homodimeric type II transmembrane C-type lectin-like receptor which is expressed on all Natural Killer (NK) cells and γδ T-cells, the majority of $CD8^+$ αβ T-cells, and a minor population of $CD4^+$ αβ T-cells with regulatory activity (32). Upon binding of corresponding NKG2D ligands (NKG2DL), human NKG2D transmits an activating signal *via* the associated transmembrane adapter protein DAP10 and the PI3-kinase pathway (33). NKG2D triggers cytolytic effector function in killer cells and co-stimulates cytokine production and proliferation in T-cells (34, 35). The ligands for NKG2D comprise an array of MHC class I-related molecules with two or three MHC class I-like domains. The human NKG2DL include MICA and MICB, and six members of the UL16-binding protein (ULBP) family that are also known as retinoic acid early transcript (RAET) proteins. In mice, the NKG2DL include five retinoic early transcript proteins (RAE-1α-ε), three variants of the H60 minor histocompatibility antigen, and the murine UL-16-binding-protein-like transcript-1 (MULT1) (36, 37). With few exceptions, normal cells do not express NKG2D ligands, but expression can be induced in many cells by "stress" including heat shock, DNA damage, viral infection, or cellular activation (36, 37). Furthermore, the NKG2D/NKG2DL system is an integral part of local immunosurveillance, as evidenced in transgenic mouse models with inducible acute up-regulation of NKG2DL (38). Interestingly, human MICA molecules can be sensed

by the Vδ1 TCR (29), but can also directly activate γδ T-cells *via* NKG2D (39). Integrated signals resulting from TCR-dependent antigen recognition and additional receptor-ligand interactions thus determine the overall γδ T-cell response (25, 40).

Regulatory functions of γδ T-cells

There is increasing evidence for multiple interactions between αβ and γδ T-cells. Activation of phosphoantigen-reactive human γδ T-cells is inhibited by conventional TCR αβ-expressing regulatory T-cells (Treg) (41, 42). Strikingly, however, it was observed that human γδ T-cells themselves can display regulatory (*i.e.* suppressive) activity on αβ T-cells (43, 44), and can antagonise the IL-2-driven expansion of Treg in mycobacteria-infected cynomolgus macaques (45). Using highly purified human CD4+ αβ T-cells and γδ T-cells, we observed under *in vitro* co-culture conditions that upon phosphoantigen stimulation, γδ T-cells suppressed the expansion of antigen-reactive CD4+ T-cells (46). Depending on the experimental conditions, Vγ9Vδ2 T-cells can thus proliferate and differentiate into cytokine-secreting and cytotoxic effector cells in response to phosphoantigens, but can also regulate αβ T-cell responses. However, γδ T-cell subsets other than the dominant Vγ9Vδ2 population also seem to be endowed with regulatory functions. Vδ1 T-cells isolated from tumour-infiltrating lymphocytes in breast cancer patients suppress αβ T-cell responses (47), and increased numbers of circulating Vδ1 T-cells were found to correlate with operational tolerance in liver allograft recipients (48). Similarly, regulation of CD4+ αβ T-cell responses has been described for murine γδ T-cells (49). Multiple additional evidence for suppressive functions of γδ T-cells has been accumulated from studies in various mouse models, not the least in γδ T-cell-deficient mice (50). Thus, it can be concluded that γδ T-cells are not only capable of recognizing antigens that are not seen by other immune cells (*e.g.* phosphoantigens) but fulfil additional important regulatory functions.

Recently, a new facet has been added by the demonstration that γδ T-cells are a major and early source of the pro-inflammatory cytokine IL-17. It has been recognised that Th17 cells represent a separate T helper cell lineage (in addition to Th1 and Th2) with an important role in the pathology of organ-specific autoimmune diseases and immunity to infection (51). Moreover, conventional (*i.e.* CD4+ TCRαβ-expressing) Th17 cells are also discussed to contribute to the initiation and perpetuation of the autoimmune response in anti-neutrophil cytoplasmic autoantibody (ANCA)-associated systemic vasculitis (52). In mouse models of autoimmunity and infection, γδ T-cells were found to share characteristic features with Th17 cells such as the expression of the chemokine receptor CCR6, the IL-23 receptor, the transcription factor retinoic orphan receptor γt (RORγt), and the aryl hydrocarbon receptor, and to provide an early and innate source of IL-17 (53, 54). Taken together, it is thus obvious that γδ T-cells despite being a numerically minor cell population in most localisations in the body, entertain multiple mechanisms to modulate innate and adaptive immune responses.

Role of γδ T-cells in vasculitis

In view of the identification of stress-inducible ligands as relevant antigens and the documented regulatory function of γδ T-cells, it is reasonable to assume that γδ T-cells contribute to the immune response in vasculitis. So far, however, only few studies have addressed this issue.

Behçet's disease

Behçet's disease (BD) is a multisystemic disease, with vasculitic lesions occurring at multiple sites including eyes, skin, joints and brain. Early studies indicated that peripheral blood γδ T-cells from BD patients responded specifically to peptides derived from the 65 kDa mycobacterial heat-shock protein (HSP) or the 60 kDa homologous human HSP; the VγVδ TCR repertoire of the responding γδ T-cells was not identified in these studies (55). Subsequently, increased proportions of γδ T-cells in the peripheral blood of BD patients were reported, which displayed signs of activation and strong production of IFN-γ and TNF-α (56, 57). Although the aetiology of BD is unknown, viral and bacterial infections have been repeatedly implicated. In addition to the described HSP reactivity of γδ T-cells, proliferative responses of BD patient's γδ T-cells to bacterial preparations have been reported (58, 59). While no specific bacterial antigen has been identified to date (with the exception of the mycobacterial HSP-65 (52)), Vγ9Vδ2 T-cells from BD patients can be activated (as expected) by microbial or synthetic phosphoantigens. Interestingly, Verjans and co-workers observed a high proportion (ranging from 15 to 29%) of γδ T-cells among T-cells present in intraocular fluid (but not in the blood) in 3 of 6 affected BD patients (60). Among these γδ T-cells, proliferation could be induced *in vitro* following stimulation with phosphoantigen IPP but not with recombinant HSP-65, pointing to the presence of Vγ9Vδ2 T-cells among the infiltrated γδ T-cells (although this was not specifically proven in this study) (60). Furthermore, peripheral blood Vγ9Vδ2 T-cells from patients with active BD were found to secrete increased amounts of the serine protease granzyme A when stimulated with phosphoantigens (61). While the studies on ocular T-cells suggested the presence of Vγ9Vδ2 T-cells (60), no preferential expression of a particular Vγ and/or Vδ chain was detected in biopsies from ulcerated oral mucosa from BD patients (62). The latter study, however, confirmed the dominance of Vγ9Vδ2 T-cells among γδ T-cells, similarly to healthy donors, also in BD patients. Furthermore, using the available anti-Vγ/Vδ mAb, Freysdottir *et al*. also analysed the presence of γδ T-cells expressing other Vγ/Vδ elements. These studies revealed alterations in the relative proportion of several γδ T-cell subsets among BD patients with different clinical conditions (62). In a recent study, Yasuoka and coworkers reported a preferential activation of peripheral blood Vδ1 as compared to Vδ2 γδ T-cells in patients with active BD (63). In the absence of functional data, however, the significance of such alterations remains unclear. Taken together, it appears that activated γδ T-cells are detected in the peripheral blood of BD

patients, and that γδ T-cells are also present locally in the affected tissue, possibly with differential prevalence of γδ T-cell subsets in oral mucosa *versus* ocular fluid. Further studies are needed to characterise the function of these γδ T-cells in more detail.

Takayasu's arteritis

Takayasu's arteritis (TA) is a chronic inflammatory panarteritis characterised by intimal thickening, fibrosis and stenosis, presumably resulting from an immune-mediated dysfunction of the arterial endothelium (64). Based on the restricted TCR repertoire, the expression of HSP-65 and MHC class II molecules in the aortic tissue and the frequent infiltration with T-cells, the aetiology is thought to involve aberrant autoimmune responses (65, 66). A large proportion (on average 30%) of leukocytes infiltrating aortic tissue in TA patients are in fact γδ T-cells (65). Moreover, increased proportions of γδ T-cells are also present in the blood of patients with active but not with inactive TA (67). Interestingly, the TCR Vδ repertoire seems to be skewed toward an over-representation of Vδ1 when compared to healthy control donors (67). So far it is unknown whether the increased numbers of Vδ1 T-cells are functionally related to the pathogenesis and/or immune regulation of TA, *e.g.* by displaying regulatory activity as shown for Vδ1 T-cells in other clinical conditions (47). It was reported that peripheral blood γδ T-cells from TA patients but not from healthy donors or SLE patients proliferate in response to recombinant human HSP-60 and kill aortic endothelial cells *in vitro* (68). Although not investigated in this study, it is possible that the HSP-60 reactivity resulted from the stimulation of Vδ1 γδ T-cells, since the dominant Vγ9Vδ2 T-cells do not respond to HSP (69). Taken together, the numerically increased proportion in the blood, the associated alterations in the expressed TCR repertoire, and the significant accumulation at the arterial lesions point to an important role of γδ T-cells in TA which deserves further investigation.

Wegener's granulomatosis

Wegener's granulomatosis (WG) is an immune-mediated disease with anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis (70). Clinical and experimental evidence supports a pathogenic role of ANCA in WG (71). Proteinase-3 has been identified as a target for humoral and cellular autoimmune responses in WG. A large range of phenotypic and/or functional alterations in the T-cell compartment has been described, which in essence all deal with αβ T-cells (52, 72). Very little information regarding γδ T-cells in WG is available. Using 5 WG patients as a control cohort for their studies on TA, Chauhan *et al*. did not observe appreciable alterations in the peripheral blood γδ T-cell compartment (67). Furthermore, increased proportions of activated CD8 T-cells co-expressing CD57 have been reported, but the TCR phenotype was not identified in these studies (73). Interestingly, γδ T-cells are present among the infiltrating cells around tubular and glomerular capillaries in the kidney of WG patients with active disease (74). There is also strong expression of MICA in the peritubular endothelium and in glomerular endothelial cells (74), pointing to a possible cross-talk of infiltrating γδ T-cells with endothelial cells. Moreover, cluster-like formations of $NKG2D^+$ and MIC^+ cells are present in WG-granulomata from the upper respiratory tract (75). Although available data do not suggest a major role for γδ T-cells in WG, it might still be informative to investigate their functional capacity in more detail.

Other types of vasculitis

In the leukoclastic form of cutaneous necrotising vasculitis (CNV) with presumed immune complex-mediated pathogenesis, a significant proportion of γδ T-cells was present in skin lesions as analysed by immunohistochemistry (76). Even higher numbers of γδ T-cells were detected in specimens from patients with documented infectious aetiology. The functions of the locally accumulated γδ T-cells are, however, unknown.

Future perspectives for γδ T-cell research in vasculitis

Apart from Behçet's disease, little attention has been paid to characterise γδ T-cells in various forms of vasculitis. In view of the expression of γδ T-cell ligands in affected tissue (68) and the observed alterations in the γδ TCR repertoire in some forms of vasculitis (64), it seems worthwhile to investigate the functions of γδ T-cells more extensively in such diseases. This might include the regulatory/suppressive capacity of isolated Vδ1 γδ T-cells in Takayasu's arteritis as well as the potential of γδ T-cells for IL-17 production in Wegener's granulomatosis and Behçet's disease.

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