# Vδ2$^{neg}$ γδ T Cells, a Multi-Reactive Tissue Subset: from Innate to Adaptive Altered-Self Surveillance

Charlotte Behr[1,2], Myriam Capone[1,2], Lionel Couzi[1,2,3], Jean-Luc Taupin[1,2,4] and Julie Déchanet-Merville[*,1,2]

[1]*Université Bordeaux 2, 146 rue Léo Saignat, 33076, Bordeaux, France*

[2]*CNRS UMR 5164, 146 rue Léo Saignat, 33 076, Bordeaux, France*

[3]*Service de Transplantation Rénale, CHU Bordeaux, 33000 Bordeaux, France*

[4]*Laboratoire d'Immunologie, CHU Bordeaux, place Amélie Rabat Léon, 33000 Bordeaux, France*

**Abstract:** Human γδ T cells are usually considered to contribute to fast-acting local immune responses. Their somewhat limited T cell receptor (TCR) diversity implies that large subsets of γδ T cells share the capacity to respond to the same restricted set of antigens, rather than showing the fine specificity toward extremely diverse antigens, as is characteristic of αβ T cells. This has been well demonstrated for Vγ9Vδ2 T cells, particularly in non-human primate models. However, much less is known about the other subsets of γδ T cells, herein collectively called Vδ2$^{neg}$ γδ T cells. Most of these cells express the Vδ1 chain, some express the Vδ3 chain, and very few express the four remaining Vδ chains (Vδ4 to Vδ8). All these Vδ chains can be associated with any of the six Vγ chains (Vγ2, 3, 4, 5, 8, 9). Vδ2$^{neg}$ γδ T cells are mainly located in epithelial tissues and the spleen, and are barely found in the circulation in normal physiological conditions. This tissue localization has limited their analysis. Establishment of murine models is difficult since murine and human γδ T cell populations vary greatly. For example, the equivalent of murine dendritic epithelial γδ T cells (DETC) does not exist in humans, and conversely, the equivalent of human Vγ9Vδ2 T cells is present only in primates. Therefore, human Vδ2$^{neg}$ γδ T cells have mostly been examined during pathological situations where their circulating levels are increased. Like Vγ9Vδ2 T cells, Vδ1 and Vδ3 T cells have been shown to be involved in widely diverse pathological contexts, such as infection, cancer, auto-immunity, and inflammation. This suggests that γδ T cells respond to a variety of altered microenvironments induced by these situations. It is acknowledged that γδ T cells can recognize ubiquitous stress-induced conserved antigens in their native form, and altered-self or foreign ligands presented on non-polymorphic molecules in total independence of classical MHC molecules. Since Vδ2$^{neg}$ γδ T cells can recognize broadly distributed antigens and are localized at the interface with the outer environment within epithelial tissues, Vδ2$^{neg}$ γδ T cells can act as a first line of defense in the surveillance of body integrity and microorganism infections. Nevertheless, Vδ2$^{neg}$ γδ T cells can also display effector/memory phenotypes similar to conventional MHC-restricted αβ T cells. This suggests an ability to mount long-lasting anamnestic immunity similar to conventional αβ T cells. Here, we will review what is currently known about Vδ2$^{neg}$ γδ T cells highlighting the pathological situations where they expand. We will also discuss what is known concerning the cellular and molecular mechanisms of their activation and their effector functions.

## A. Vδ2$^{NEG}$ γδ T CELL REPERTOIRES DURING NORMAL PHYSIOLOGICAL CONDITIONS

Although γδ T cells comprise a small portion of circulating lymphocytes at birth, Vδ2$^{neg}$ γδ T cells comprise the majority [1]. The absolute count of total γδ T cells drops sharply during the first years of life [2], mostly due to a decrease in the Vδ2$^{neg}$ γδ T cell population. The Vδ2 T cell population actually remains stable or tends to increase in the peripheral blood from birth to the adults who exhibit a ratio of Vδ1:Vδ2 much less than 1. Dramatic variations in γδ T cell populations are observed between individuals, and inversion of this ratio is frequently observed in normal blood donors. These variations suggest that genetic and/or environmental factors are involved in shaping γδ T cell populations.

To get further insight into this issue, several studies have investigated the repertoire of γδ T cells in adult peripheral blood and in cord blood. The diversity of Vδ1 T cells was found to be very restricted as compared to conventional T cells, but wide disparities between individuals were noted [3-5]. It is still unknown as to why γδ T cell repertoires vary so greatly (from sharply clonal to polyclonal) among normal subjects. While the adult repertoire might have been shaped by exposure to a myriad of environmental stimuli, the sheltered status of the fetus is expected to illustrate an immature repertoire of γδ T cells. However, the repertoire of Vδ1 and Vδ2 T cells was also found to be restricted in normal newborns [3]. Evaluation of newborn identical twins showed differences in the repertoire of Vδ1 and Vδ2 T cells,

*Address correspondence to this author at UMR CNRS 5164, Université de Bordeaux 2, Bat 1B, 1$^{er}$ étage, 146 rue Léo Saignat, F-33076 Bordeaux Cedex, France ; Tel: +33-557571471; Fax: +33-557571472;
E-mail: Julie.dechanet@u-bordeaux2.fr

1874-2262/09 2009 Bentham Open

excluding a genetic explanation for Vδ1 T cell oligoclonality.

Similar conclusions can be drawn from the analysis of the phenotype of newborn blood γδ T cells. In contrast to αβ T cells, Vδ1 T cells, and particularly Vδ2 T cells, include a relatively high proportion of cells that do not express a naïve phenotype (CD45R0$^{-}$/CD11a$^{dull}$/CD27$^{+}$) suggesting their *in utero* activation [2]. Here again, inter-individual variations are wide. Because this priming would take place before any "strong" environmental exposure, these cells could be responding to self antigens. An alternative explanation could include the low grade exposure to non-pathogenic and ubiquitous environmental elements able to cross the placental barrier from the mother to the fetus.

## B. PHYSIOLOGICAL LOCALIZATION OF Vδ2$^{NEG}$ γδ T CELLS IN TISSUES

Vδ1 T lymphocytes represent the first γδ T cell subset to emigrate from the fetal thymus. Vδ1 T cells are guided to tissues, particularly epithelial tissues where they are found enriched, by the expression of appropriate homing receptors. Vδ2$^{neg}$ γδ T cells are poorly represented in secondary lymphoid organs, where Vδ2 T cells are the dominant γδ T cell population. However, in the spleen Vδ2$^{neg}$ γδ T cells are preponderant and represent about 15% of splenic T cells. They express mainly the Vδ1 chain and CD8, and are located in the marginal zone and the red pulp [6, 7]. Vδ2$^{neg}$ γδ T cells are the predominant γδ T cell subset in normal human epithelial surfaces, with selective accumulation in the epithelium of the large intestine [8, 9], and in the skin. Skin Vδ2$^{neg}$ γδ T cells express the homing receptors CCR8 and cutaneous lymphocyte-associated antigen (CLA) [10,11] and express a restricted repertoire which is distinct from the repertoire in the peripheral blood [12]. In these tissues, they appear scattered as single cells, likely reflecting their resident status, rather than being a localized T cell infiltration. The Vδ3 T cell subset represents and average of 18% of intra-epithelial γδ T cells [13]. γδ T cells expressing the Vδ4-Vδ8-chains can comprise a significant fraction of the mucosal δ-chain T cell receptor repertoire in some individuals [5]. The intestinal Vδ1 repertoire in the small intestine and colon appears compartmentalized, and shows no overlap with the circulating Vδ1 repertoire. Dominant Vδ1 transcripts differ between the small intestine and colon, and vary between individuals. These differences suggest that Vδ2$^{neg}$ γδ T cells might be specialized to recognize antigens specifically expressed in epithelial tissues evocating a role as a first-line defense in mucosal immunity [5,13-15]. Vδ2$^{neg}$ γδ T cells comprise approximately 20% of the mononuclear cells found in early pregnancy deciduas, and preferentially express the Vδ1 chain and co-express CD56 and IL7R [16].

## C. PATHOLOGICAL CONTEXTS OF Vδ2$^{NEG}$ γδ T CELL ACTIVATION

The pathological contexts in which involvement of Vδ2$^{neg}$ γδ T cells have been reported are numerous. Vδ2$^{neg}$ γδ T cells can be involved in inflammatory and autoimmune diseases, wound healing, carcinomas and some blood malignancies, as well as bacterial, viral, and parasitic infections. In most cases, repertoire analysis showed important changes in the Vδ2$^{neg}$ γδ T cell populations found in tissues and blood. The pathologies that activate Vδ2$^{neg}$ γδ T cells are often different from those that activate Vδ2 T cells, illustrating the different roles these populations play due to different antigen specificities and homing capacities.

### 1. Cancer

Given their high levels in epithelial tissues, Vδ1 γδ T lymphocytes might function as a first line of defense against epithelial malignancy. In line with this assumption, Vδ1 T cells, and to a lesser extent other Vδ2$^{neg}$ γδ T cells, were found to infiltrate epithelial tumors from various origins, including melanomas [17], colon adenocarcinomas [9], renal carcinomas [18,19], and lung adenocarcinomas [20,21]. γδ T lymphocytes infiltrating renal cell carcinomas were found to express activation markers, suggesting previous activation *in vivo* [22]. Along the same line, γδ tumor infiltrating lymphocytes (TIL) from adenocarcinomas of the lung could be selectively expanded *in vitro* without additional stimuli [20]. Interestingly, the proportion of Vδ1 T cells among intraepithelial γδ T cells increased up to 50% in tumors that were positive for MICA/B [23]. Therefore, these molecules might contribute to Vδ1 T cell activation and expansion (see below). In line with their putative anti-tumor potential, activated Vδ1 TILs were found to display a strong cytotoxic activity against autologous, as well as heterologous, carcinoma cells [18,23] (see below).

Several observations suggest a TCR-driven selection of γδ T lymphocytes infiltrating epithelial tumors. A particular Vγ3Vδ1-expressing clone was observed in 3 samples of cultured TILs from a patient with recurrent renal cell carcinoma over a period of 3 years [18]. In *ex-vivo* analyses, a higher Vδ1:Vδ2 ratio was found in TILs, when compared to peripheral blood lymphocytes (PBLs) from renal cell carcinoma patients. Furthermore, differences in the Vδ repertoire between TILs and PBLs confirmed previous studies [24], and suggest a tumor-dependent reshaping of the local γδ TCR repertoire [19]. The prominence of Vδ1 T cells in carcinomas might also be linked to their different homing capacities due to the expression of adhesion molecules and chemokine receptors that are different than those on Vδ2 T cells [25]. The preferential accumulation of Vδ1 T cells, as compared to the Vδ2 T cell subset, in the tumor compartment of esophageal cancer patients might be favored by the larger array of adhesion molecules, such as CD49d, CD49e and $\alpha_E\beta_7$, utilized to bind to squamous carcinoma cells [26].

### 2. Autoimmune and Inflammatory Diseases

Vδ2$^{neg}$ γδ T cell populations are expanded in several autoimmune and chronic diseases, but their role in the physiopathology of these diseases is not clear. Numerous reports have analyzed the relative numbers of Vδ2 versus Vδ1 T cells, as well as the γδ TCR repertoire, in the peripheral blood and target tissues.

#### *2.1. Arthritis*

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation of the synovium, including massive T cell infiltration. Activated γδ T cells preferentially expressing the Vδ1-chain have been reported to be present in arthritic infiltrate. These cells display an activated phenotype, and a polyclonal Vδ-chain repertoire

with extensive junctional diversity, two features which differ from their circulating counterparts [27, 28].

In Lyme arthritis, a disease caused by the spirochete *Borrelia burgdorferi*, recruitment of Vδ1 T cells has been described in the synovial fluid. These recruited Vδ1 T cells proliferate *in vitro* in response to *Borrelia burgdorferi* lysate, whereas circulating Vδ1 T cells from either patients suffering from Lyme arthritis or unexposed donors do not have a proliferative response. These responding Vδ1 T cells utilized various Vγ chains, although Vγ8 was preferentially used [29]. Moreover, Vδ1 T cell clones derived from Lyme arthritis synovial fluid show diverse delta-chain CDR3 length and junctional sequences, all of which respond to *Borrelia burgdorferi* lipoproteins (see section on "target cells" below). Taken together, these data suggest Vδ1 T cells recognize self-antigen and are specifically recruitment to the inflamed synovium.

### *2.2. Inflammatory Bowel Diseases*

Celiac disease is an auto-immune bowel disease characterized by an inflammatory reaction due to gluten intolerance, leading to villous atrophy and crypt proliferation. In patients suffering from this disease, expansion of Vδ1 T lymphocytes has been described in the inflamed mucosa [30]. This expansion is associated with an activated phenotype and junctional diversity [31, 32]. In Crohn's disease, another inflammatory bowel disease, local expansion of Vδ1Vγ8 T cells associated with granulomas has been described. In addition, an increase of Vδ1 T cells was detected in the blood of these patients [33, 34]. Repertoire studies revealed that oligoclonal expansion of Vδ1 T cells from inflamed mucosa was rare, but rather several patients had highly diversified repertoires [35]. However, even during non-pathological conditions, the repertoire of Vδ1 T cells has been shown to differ depending on the intestinal region (duodenum, jejunum or ileum), as well as between sites within the same colonic region. Thus, specific recruitment of auto-reactive Vδ1 T cell clones in the inflamed tissues is difficult to identify. This does not exclude that such locally expanded resident Vδ1 T cells could contribute to the tissue damage, as they may recognize self antigen(s) expressed under physiological conditions. Expression of the same array of antigens might be locally increased due to inflammation, leading to local proliferation of γδ T cells, with no major change in their repertoire. In this condition, the observed diversified repertoire would result from degenerative recognition of self-antigens. Alternatively, those cells might be recruited as bystanders of an on-going inflammatory reaction and become activated by TCR-independent mechanisms, involving NKG2D-MICA interactions (as shown for $CD8^+$ αβ T cells in celiac disease [36]), or by chemokines secreted by other inflammatory cells. Finally, Vδ1 T cells may not be involved in tissue damage, but rather play a major role in tissue repair or immunoregulation to facilitate the return to homeostasis. In this respect, a subset of small intestinal $CD8^+TCR\gamma\delta^+NKG2A^+$ IELs have been shown to display immunoregulatory properties in patients with celiac disease by suppressing the cytotoxic activity of $CD8^+$ αβ T cells, through the production of TGF-β1 [37].

### *2.3. Cutaneous Lesions*

During systemic sclerosis, a significantly increased prevalence of Vδ1 T cells is observed in blood and skin [38]. Skin Vδ1 T cells were detected mainly in the perivascular areas during the early stage of the disease, and to a lesser extent during the later sclerotic phase. This suggests that Vδ1 T cells may respond to skin lesions by local expansion in response to a self-antigen, or by passive recruitment in response to inflammatory chemokines produced by the inflamed endothelia. These cells display an activated cytotoxic phenotype and express CD49d. Activated Vδ1 $CD49d^+$ T cells might bind to VCAM-1 expressed on inflamed vascular endothelial cells and directly damage endothelium. Moreover, CD49d can also bind to collagen I and fibronectin, allowing Vδ1 T cells to accumulate in the perivascular connective tissue. A recent report showed that the epidermal Vδ1 T cell resident population plays a critical role in skin homeostasis through the release of IGF-1, an insulin growth factor that regulates keratinocyte migration and contributes to wound epithelialization [11]. In addition, a comparative analysis of functional capabilities of Vδ1 T cells isolated from chronic and acute wounds, demonstrated that Vδ1 T cells from chronic wounds are functionally impaired in the production of IGF-1 and are less responsive to TCR activation. These data are the first demonstration that Vδ1 T cells play a role in wound healing and make them attractive targets for chronic wound therapy.

In infectious lesions, such as localized cutaneous leishmaniasis (LCL) and leprosy lesions, immuno-histochemical analyses have identified the presence of both Vδ2 and Vδ1 T cells [39]. The two γδ T cell populations occupy distinct, but overlapping, environmental niches. Vδ1 and Vδ2 T cells were both detected in dermal granulomas, but Vδ2 T cells were predominant. In contrast, Vδ1 T cells were clearly predominant in the epidermal infiltrate. Interestingly, unlike the clonal diversity described in the blood of these patients or in normal skin, limited diversity and over-representation of certain TCR sequences were described in the lesions, likely reflecting local oligoclonal expansion. The major junctional sequences were also shown to vary between microanatomical sites, suggesting a few clones from resident Vδ1 T cells expanded in each microanatomical interstitium [40]. Because both *Leishmania* and *Mycobacteria* produce phosphoantigens derived from the non-mevalonate pathway, it is likely that Vδ2 T cells were recruited to the site of microorganism replication and then locally expanded. The resident Vδ1 T cells may respond to self-antigens that were upregulated due to microorganism products or as a consequence of inflammatory responses. The Vδ2 T cell subset might be largely responsible for containment and elimination of the microorganism, whereas the Vδ1 T cell subset might be involved in the initiation and/or resolution of the granuloma, as well as in tissue repair.

## 3. Viral Infections

The contribution of γδ T cells to the host response against viruses has been explored in a large number of human viral infections (reviewed in [41-43]). In these studies, the

involvement of γδ T cells was supported by the observation that γδ T cells are increased in the blood and/or in the infected organs of the patients. Two RNA viruses (HIV and HCV) and three herpes viruses (EBV, HHV8 and CMV) caused expansion of Vδ2$^{neg}$ γδ T cells. The localization of Vδ2$^{neg}$ γδ T cells in intestinal, genital and respiratory mucosal epithelia makes these cells an important component of the first-line of defense at the entry site of most of these pathogens.

### *3.1. RNA Viruses*

During HIV-1 infections, increases in the percentage and/or absolute number of circulating Vδ1 T cells have been reported within blood [44-46], bone marrow [47] and rectal mucosa [48]. This apparent expansion of Vδ1 T cells is enhanced due to the anergic state of Vγ9Vδ2 T cells, which actually decrease in number [41,43]. Vδ1 T cells from HIV patients do not express particular Vγ chains, nor do they exhibit any evidence of clonal selection, when compared to healthy donors [49,50]. However, they present a peculiar phenotype with markedly enhanced expression of CD103, CCR9, HLA-DR, CD45-RO, CD94, NKG2C CD158a/h and CD158b/j, which might be the result of a chronic activation of Vδ1 T cells in HIV-infected individuals [47,48,51,52]. CD103 and CCR9 expression suggests these cells originate from the intestine, and that the perturbation of the intestinal mucosa associated with HIV infection may favour either mucosal depletion or proliferation and recirculation of Vδ1 IEL. The dysfunction of the intestinal barrier due to local depletion of CD4$^+$ T cells in chronic HIV patients, and the resulting bacterial translocation through the damaged epithelium [53], could also favour Vδ1 T cell expansion, as it has been shown during mucosal injury in mice [54].

Expansion and activation of Vδ1 T cells have been observed in patients infected with Hepatitis C virus (HCV). Vδ1 T cells represent the major subset of γδ T cells infiltrating the liver and produce functional levels of IFNγ after polyclonal activation *in vitro* [55,56]. Compartmentalization of Th1 Vδ1 T cells in chronically infected tissue could contribute to necroinflammatory liver disease. Comparison of Vδ1 distribution in normal and HCV-infected livers has not yet been assessed and there is no evidence for a specific recruitment of this subset in the infected organ. Like patients infected with HIV, patients with chronic HCV have a decreased percentage of circulating Vγ9Vδ2 T cells, which may reflect their recruitment to inflamed compartments and/or altered cellular immune responses [57].

### *3.2. CMV and Other Herpes Viruses*

Human cytomegalovirus (CMV) is a widespread beta herpes virus that can persist lifelong in absence of symptoms in immunocompetent individuals. Adaptive T lymphocytes are recognized to play a crucial role in the control of CMV infections [58-60]. In contrast, CMV can be life-threatening for immunologically immature or compromised individuals, such as neonates, AIDS patients or transplant patients. CMV infections in these contexts can lead to pneumonia, hepatitis, colitis or retinitis.

γδ T cells likely play a large role in CMV infections of such immuno-compromised patients. We have described a major and long-lasting expansion of γδ T cells in the peripheral blood of kidney transplant patients during CMV infection [61]. The percentage of γδ T cells was found to exceed up to 40% of circulating T cells in some patients and remained stable for years post-infection. Similar expansions have also been observed in liver, lung, heart or intestine transplant patients (our unpublished results). A TCR repertoire analysis of these cells indicated that Vγ9Vδ2 T cells were not affected by CMV-infection, whereas Vδ1 T cells, and to a lesser extent Vδ3 T cells, made up the majority of the expanded cells [62]. No selection for a particular Vγ-chain was noticed. Additionally, in one lung transplant patient with a CMV infection, Vδ5 T cells represented 25% of all circulating T cells [63]. This expansion of Vδ2$^{neg}$ γδ T cells is a striking hallmark of CMV infection in immuno-suppressed patients, as it has not been detected in transplant patients infected with bacteria, fungi, mycobacteria, parasites or other viruses [61].

Interestingly, the Vδ chain CDR3 diversity of Vδ2$^{neg}$ γδ T cells is more restricted in CMV-infected patients, than it is in non-infected transplant patients. This could be due to antigenic selection *in vivo* [62]. Some patients display monoclonal populations of Vδ1, Vδ3 or Vδ5 T cells, reaching up to 30% of the total circulating T cells [62, 63]. These observations illustrate vigorous selection and expansion of certain populations of γδ T cells during CMV infections.

Remarkably, a similar association between CMV, immunosuppression and Vδ2$^{neg}$ γδ T cell expansion has been identified in immunodeficient children with a hypomorphic mutation in the gene encoding the Recombinant Activating Gene-1 protein (RAG-1). RAG-1 encodes the recombinase responsible for V(D)J recombination, which is a required step in B and T lymphocyte development. While defects in RAG-1 or -2 lead to a complete T and B cell deficiency, hypomorphic mutations in these genes are characterized by residual T and B cell differentiation. Most of the children with recessive hypomorphic RAG1 mutations present oligoclonal expansions of Vδ1, Vδ2 (but Vγ9$^-$), Vδ3 or Vδ5 γδ T cells, a sharp deficit in αβ T cell numbers and severe complications during CMV infections [64, 65].

Activation and expansion of Vδ2$^{neg}$ γδ T cells as a signature of CMV infection is not restricted to immunocompromised patients. Indeed, CMV-seropositive, as compared to CMV-seronegative, healthy blood donors display a slight, but significant, increase in their percentage of Vδ1 T cells (or Vδ3 T cells in some individuals), while Vγ9Vδ2 T cells remain unchanged. Amazingly, the phenotype of the expanded cells (Vδ1 or Vδ3 T cells) is shifted from a naive to an effector-memory profile characterized by the CD27$^-$CD45RA$^+$ phenotype (TEMRA phenotype). In addition, the repertoire of Vδ1 T cells is more restricted [66]. All of these features underline the unique impact of CMV on Vδ2$^{neg}$ γδ T cells, as CMV contact (assessed by serology) is necessary and sufficient to engage Vδ2$^{neg}$ γδ T cells toward the TEMRA phenotype. Thus, CMV is one major environmental factor linked to the restricted repertoire of Vδ2$^{neg}$ γδ T cells previously reported (see paragraph A).

These observations argue for a striking response of Vδ2$^{neg}$ γδ T lymphocytes to CMV infection, in both immuno-suppressed and fully immuno-competent individuals. Immuno-suppression is not necessary for a local γδ T cell expansion in

response to CMV infections. However, a compromised αβ T-cell population in immunocompromised patients probably induces an altered homeostatic balance in the periphery, and therefore an exacerbated and more sustained expansion of Vδ2$^{neg}$ γδ T cells. The expansion of Vδ2$^{neg}$ γδ T cells could serve as a new and reliable marker of CMV infection in allorecipients under immunosuppressive regimens.

Vδ2$^{neg}$ γδ T cells also play a role in other herpes virus infections. Recently, a study showed that HHV8-infected immunocompetent individuals (seven patients and five controls) have expanded Vδ1 T cell populations with reactivity toward HHV8-infected cells *in vitro* [67]. A broader study including a larger subject population and Vδ1 T cell repertoire analysis is needed to determine if this expansion is antigen-driven. Additionally, Vδ1 T cells reactive against EBV-transformed B lymphoblastoid cell lines have been reported to be enriched in the peripheral blood and synovial tissue of reactive arthritis patients [68]. The role of EBV in this expansion is unlikely as the same Vδ1 T cells are also able to recognize uninfected activated B cells (see section on "target cells").

## D. STIMULATION OF TARGET CELLS FOR Vδ2$^{NEG}$ γδ T CELLS IN THESE DIFFERENT CONTEXTS

Collectively, the observations summarized above suggest that the Vδ2$^{neg}$ γδ T cell population is comprised of multiple different populations. Some of these cells are resident in the epithelia and are equipped to swiftly respond to local pathogenesis. Others are circulating cells that can preferentially home to epithelia altered by cell transformation, chronic inflammation, or tissue damage, possibly associated with an infectious agent. As such, Vδ2$^{neg}$ γδ T cells are likely involved both in rapid local immune responses and in later regulatory and tissue repair activities. In the following section, we will review the cellular and molecular activators and targets of the Vδ2$^{neg}$ γδ T cells, with particular emphasis on the CMV infection context.

The cellular targets of Vδ2$^{neg}$ γδ T cells have been identified within most of the diverse pathological contexts cited above (Fig. **1**). Weakly-specific complex pattern of molecules linked to the alteration of target cells due to transformation, infection or PAMP (pathogen associated molecular pattern) mediate the recognition. Propensity to autoreactivity makes γδ T cells potentially harmful cells, thus requiring tightly controlled activation. This control is achieved through appropriate combination of TCR-ligand interactions, co-stimulatory molecule interactions, down-regulation of inhibitory ligands and recognition of secreted stimulatory cytokines.

### 1. Dendritic Cells (DC) Activated by Microbial Components

Activation of Vδ1 T cells by microbial lipids has been proposed. Das *et al.* derived two Vδ1 T cell lines from healthy blood donors. The clones were selected based on their proliferative response to monocyte-derived DCs loaded with total lipid extracts from Gram-negative bacteria or LPS [69]. The presence of DCs was required, but none of the known major antigen presenting molecules was involved. Although proliferation was blocked with an anti-Vδ1 specific antibody, reporter cells transfected with TCR from responding clones failed to respond. This suggests that Vδ1 T cells indirectly recognize DC-presented lipids. DCs are initially activated by lipid components through a Toll-like receptor (TLR)-dependent pathway, resulting in the expression of an unknown ligand recognized by the Vδ1 TCR. Complete activation of Vδ1 T cells then requires concerted adhesion/costimulation by CD2/LFA3, ICAM/LFA1, NKG2D/MICA, and IL12. The identity and nature of the ligand remains to be determined. It is possible that this ligand is an induced self-antigen recognized in its native form, or a molecule which presents microbial lipids or altered-self products.

In line with these observations, clones derived from Vδ1 T cells infiltrating the synovial fluid of Lyme arthritis patients were shown to proliferate in response to lipoproteins from a sonicated fraction of *Borrelia burgdorferi* in the presence of metabolically active dendritic cells [70]. Stimulation by *B. burgdorferi*-pulsed dendritic cells is not dependent on class I or II MHC or CD1 molecules. Stimulation is abrogated by blocking with an anti-γδ TCR antibody and results from an indirect activation of monocytes or DCs *via* TLR and caspase-8 dependent pathways [71, 72]. This process is not specific for *B. burgdorferi*, as DCs activated by ligands for TLR2, TLR3, TLR4 and TLR9 are able to activate synovial fluid Vδ1 T cells [71]. This is likely following the same type of indirect mechanism involving APC activation that *Onchocerca volvulus* extracts induces stimulation of blood Vδ1 T cells [73, 74]. These findings suggest that Vδ1 T cells do not directly recognize ligands from these pathogens, but recognize undefined self-antigen(s) presented on altered-self cells or antigen presenting cells, such as DCs.

### 2. Tumour Cells

Numerous studies have shown that activated γδ T cells can kill cancerous epithelial cells. In contrast to freshly isolated skin T cells, most skin-derived Vδ1 T cell clones were shown to express detectable levels of perforin, and were able to kill SK-Mel2 and HS-294 melanoma cell lines [10]. The predominant Vγ3Vδ1 TIL clone isolated from three different tumors from a single patient with renal cell carcinoma (described above) kills autologous tumor cells [18]. The γδ TILs that infiltrated lung cancer could recognize tumor cells expressing the monomeric laminin receptor. Only Vδ1 T lymphocytes, however, were capable of selective lysis of autologous tumors expressing this receptor [75]. Human intestinal Vδ1 TILs displayed strong cytotoxic activity against autologous, as well as heterologous carcinomas [9, 23]. In most of these studies, abrogation of target cell killing was neutralized by anti-TCR antibodies, thus suggesting antigenic recognition of the transformed targets by Vδ1 T cells. The help of co-stimulatory molecules, such as MICA, β2- and β7-integrins, and the fibronectin receptor is also required to induce Vδ1 T cell cytotoxicity [9, 23].

Increasing evidence argue for a potential anti-tumor role for Vδ1 T cells against leukemia and lymphomas. This activity was previously proposed to be a unique property of Vγ9Vδ2 T cells. Vδ2 T lymphocytes can infiltrate and kill numerous epithelial tumors, suggesting there are not distinct anti-tumor specificities for each γδ T cell subtype.

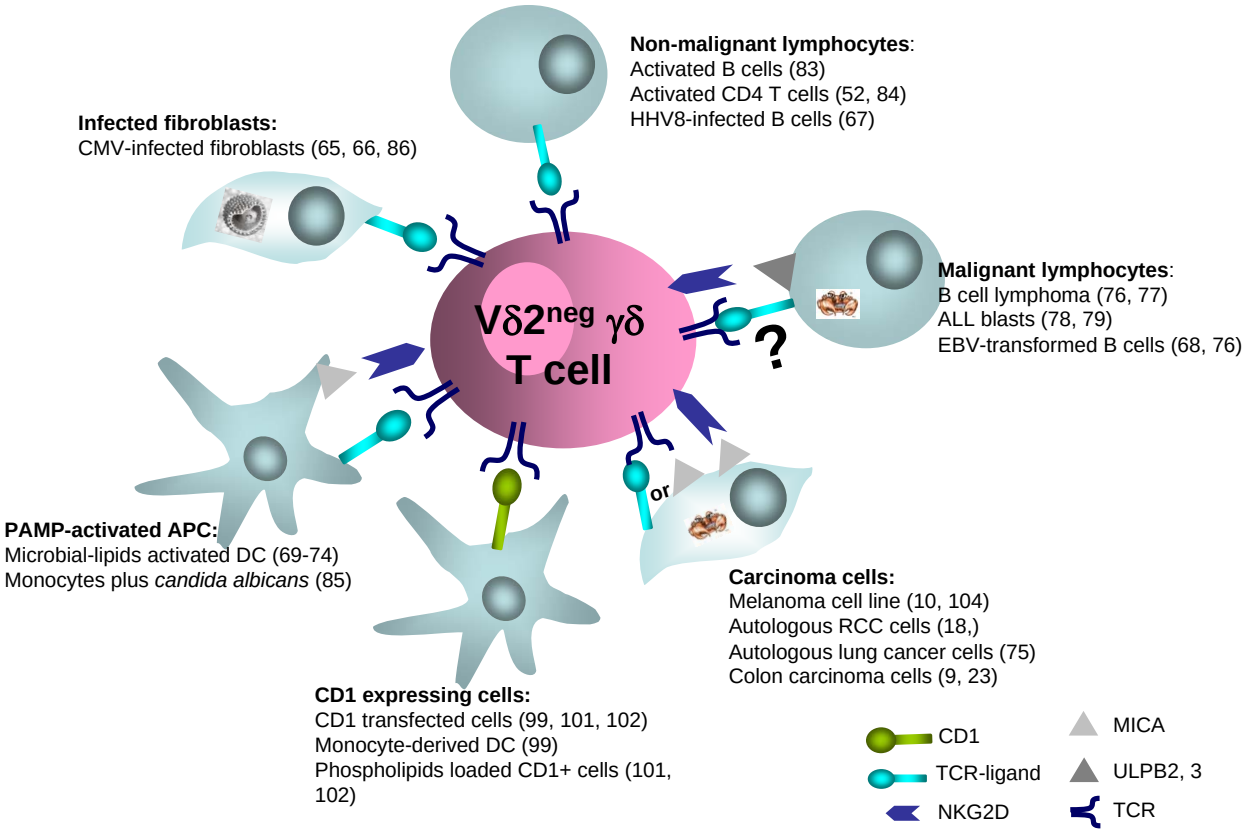


**Fig. (1).** The different cell types known to stimulate Vδ2$^{neg}$ γδ T cells.

Additionally, peripheral blood Vδ1 T cells can recognize B-cell lymphomas [76, 77]. Donor-derived Vδ1 T cell lines isolated from bone marrow transplant recipients with acute lymphoid leukemia, were cytotoxic *in vitro* toward lymphoid cell lines and primary leukemia blasts, whereas myeloid cell lines were not killed [78, 79]. Interestingly, in low-grade follicular non-Hodgkin lymphoma patients [80] and B-chronic lymphoid leukemia patients [81], tumor cell expression of ULBP2 or ULBP3 is associated with a better killing of autologous cells by NKG2D-expressing Vδ1 T cells *in vitro* and a better prognosis.

In conclusion, Vδ2$^{neg}$ γδ T cell tumor-induced activation is a complex process involving TCR-dependent and -independent signals and co-stimulatory signals, which leads to proliferation and effector anti-tumor responses, resulting in cancer cell killing and cytokine release. Stress signals delivered during malignant transformation likely lead to both TCR-recognized altered auto-antigens and co-stimulatory factors, such as NKG2D-ligands. The remodeling of TCRδ repertoire expressed by anti-tumor Vδ2$^{neg}$ γδ T cells implicates the CDR3δ loop in antigen recognition, as was reported recently for Vδ2 T cells [82]. This finding is better documented in the context of epithelial tumor cells, rather than with malignant blood cells.

## 3. Infected Cells

Multiple reports have examined the response of Vδ1 T cells to EBV-transformed B lymphoblastoid cell lines *in vitro* through TCR- and LFA1-dependent pathways [68, 76, 83]. However, a role of viral antigens in this activation has been ruled out, since the response of lymphoblastoid cell-reactive Vδ1 T cells can also be elicited by normal activated B cells.

Vδ1 T cells from HIV-1-infected patients are cytotoxic against HIV-1-infected, but also uninfected, autologous or allogeneic CD4 T cells [84]. This effect is probably independent of TCR engagement, as it involves increased HLA-E expression on HIV-infected or activated CD4 T cells and the triggering of the HLA-E-receptor NKG2C on Vδ1 T cells [52]. Highly cytolytic Vδ1 T cells might serve a protective anti-viral function by killing infected CD4 targets, but they might also be involved in the HIV-associated immunopathogenesis by contributing to the depletion of bystander CD4 T cells. More recently, a subset of Vδ1 T cells from non-progressor HIV-1-infected patients was shown to produce IFNγ and IL17 in response to *Candida albicans in vitro* [85]. These cells, which surprisingly displayed a central memory phenotype, might play a role in the control of opportunistic infections in these patients through recirculation *via* lymph nodes and peripheral tissues.

Vδ1 T cells from HHV8-infected individuals are reactive against HHV8-infected cell lines. The recognition is blocked by an anti-CD3 antibody, thus implicating TCR recognition.

Through their production of IFNγ, Vδ1 T cells are able to prevent the release of infectious viral particles from the infected cell lines, indicating that they could play a role in the anti-viral response directed against HHV8 [67].

## 4. Cross-Reactivity Against CMV-Infected Cells and Tumour Cells

A number of Vδ1, Vδ3 and Vδ5 T cell clones, expressing diverse Vγ chains, derived *in vitro* from several CMV-infected transplant patients or healthy donors, are reactive towards CMV-infected cells *in vitro*, whereas Vγ9Vδ2 T clones are not reactive [66, 86]. Vδ2$^{neg}$ γδ T lymphocytes exhibit a potent cytotoxic activity, killing CMV-infected targets and limiting CMV propagation *in vitro* [86]. This cytotoxic potential is mediated through granzyme and perforin release. Cells infected by non-CMV herpes viruses, such as VZV, HSV, or EBV, are not recognized by Vδ2$^{neg}$ γδ T cells. This recognition of target cells does not involve MHC class I molecules or NKG2D. Vδ3 T cell clones isolated from a hypomorphic RAG1 mutation patient produce TNFα when cultured with CMV-infected cells [65]. These results demonstrate that Vδ2$^{neg}$ γδ T cells can play a protective role against CMV infection and they represent a functionally distinct population from Vγ9Vδ2 T cells (Fig. **2**).

Strikingly, the CMV-infected cell-reactive Vδ2$^{neg}$ γδ T cell clones isolated from CMV-infected transplant recipients, also displayed cytotoxic activity against CMV-uninfected intestinal (HT29, CaCo2) or other (HeLa) epithelial tumor cell lines, but not against normal epithelial cell lines [86]. This ability to recognize altered-self (infected or transformed) cells is reminiscent of the Vγ9Vδ2 T cells' ability to recognize both tumor cells and cells infected by microorganisms, such as mycobacteria or plasmodium. This is also consistent with the preferential homing of Vδ2$^{neg}$ γδ T cells to intestinal epithelia and their abundance among carcinoma-infiltrating T lymphocytes [23]. Relatively high frequencies (around 10%) of Vδ2$^{neg}$ γδ T lymphocytes with dual anti-CMV and anti-tumor specificity were found among polyclonal γδ T cell lines from CMV-infected transplant patients [86], demonstrating their important physiological relevance (Fig. **2**).

The recognition of CMV-infected cells and tumor epithelial cells by Vδ2$^{neg}$ γδ T cells isolated from CMV-infected patients is TCR-mediated, as shown by inhibition with anti-TCR antibody and by TCR-internalization [86]. Direct involvement of the TCR was demonstrated by specificity transfer. TCR-deficient JRT3 Jurkat cells transfected with two different Vδ2$^{neg}$ γδ TCR, Vδ1Vγ9 or Vδ5Vγ4, but not with Vδ2Vγ9 restored tumor cell lines recognition recapitulating the recognition by the parental clones (our unpublished results). By immunizing mice with HT-29 tumor cells, a monoclonal antibody directed against both CMV-infected cells and tumor cells was generated. It is able to block Vδ2$^{neg}$ γδ T cell clone reactivity against both target cells. It is also able to abrogate Vδ2$^{neg}$ γδ TCR-transduction, demonstrating that the monoclonal antibody recognizes a TCR ligand present on both cell targets

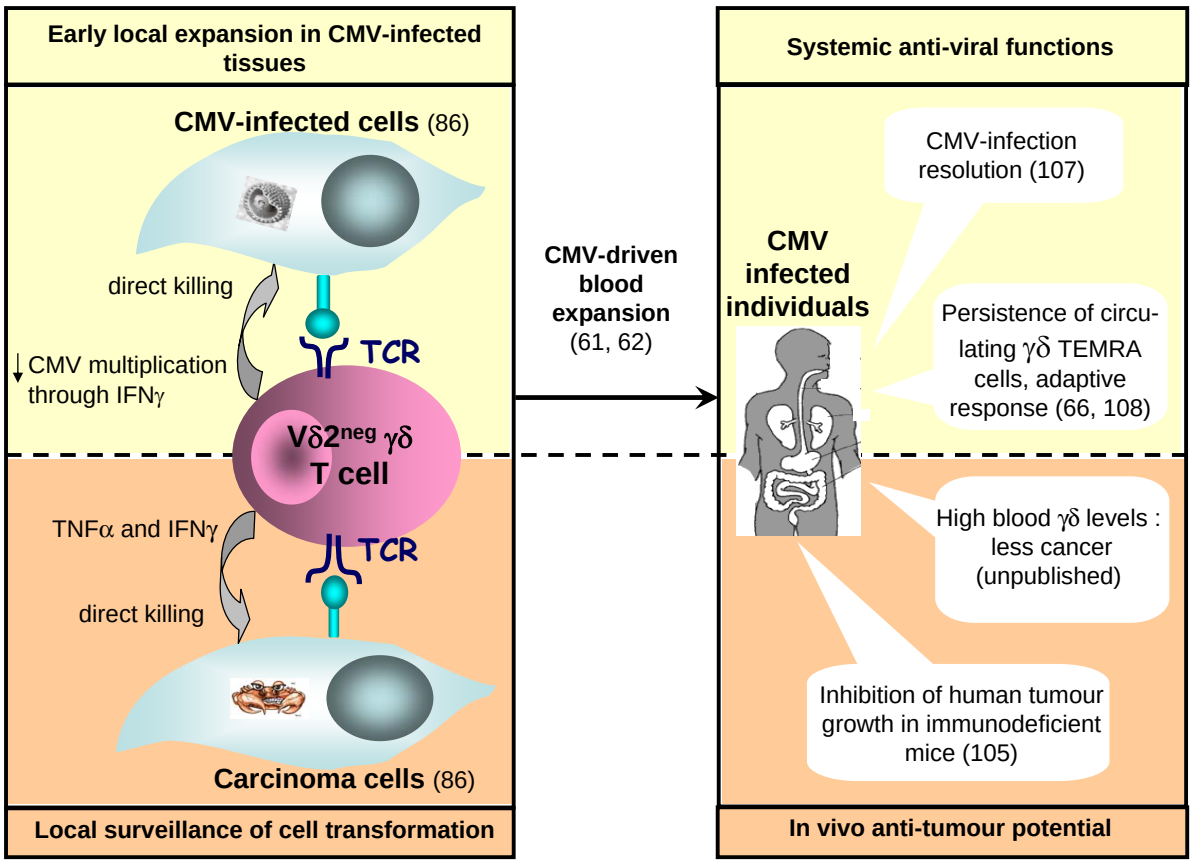


**Fig. (2).** Shared anti-viral and anti-tumoral reactivity of Vδ2$^{neg}$ γδ T cells.

(manuscript in preparation). The identity of this ligand is unknown, but loss of staining following proteinase treatment of target cells suggests it is a protein.

## E. TCR LIGANDS OF Vδ2$^{NEG}$ γδ T CELLS

In several of the pathogenic situations stressed above, Vδ2$^{neg}$ γδ T cells expressing different Vγ or Vδ chains with different junctional regions, recognize identical targets, such as tumor cells and/or infected cells. This suggests a degenerative mode of recognition where very diverse γδ TCRs are able to recognize either diverse ligands on altered-self cells or altered-self cells expressing a conserved, yet still undefined, ligand for Vδ2$^{neg}$ γδ T cells. The limited germ-line diversity of the γδ TCR due to the small number of used gene segments encoding the Vδ and Vγ regions taken with the recognition of the target in a classical MHC-molecules (HLA I and II) independent manner, led to the suggestion that γδ T cells might recognize non-polymorphic antigen-presenting molecules. Likewise, all the molecular ligands for Vδ1 T cells identified so far are non-polymorphic MHC related molecules i.e. MICA/B and CD1a, CD1b, CD1c, CD1d (Fig. **1**). These candidate ligands have been proposed on the basis of experiments aimed at inducing Vδ2$^{neg}$ γδ T cell reactivity through transfection with the considered molecule and/or by means of Vδ2$^{neg}$ γδ TCR transfer experiments (see below). However, cognate interactions between a Vδ2$^{neg}$ γδ TCR and specific ligands have not yet been reported.

### 1. MICA

One of the first ligands reported for the Vδ1 TCR was MICA. This molecule, as well as MICB, belongs to a family of molecules resembling the classical MHC class I proteins (class Ib molecules). MICA and MICB do not have murine orthologs. This family also includes five retinoic acid early inducible-1 proteins: the four RAET1 family members (I, H, N, E), formerly named UL-16 Binding Proteins (ULBP1 to 4, respectively), and RAET1G. The surface expression of these proteins is induced or enhanced under stress or inflammatory conditions, such as cellular transformation, heat shock, treatment with DNA-damaging agents, and bacterial or viral infections. The TLR activation pathways often function to upregulate these molecules [87]. These ligands share a common receptor, NKG2D, which is expressed on all γδ T cells and is a major co-stimulatory molecule. NKG2D can also function alone to induce full activation, as reported in celiac disease for intraepithelial αβ CD8 T cells [36, 88]. The role of NKG2D in γδ T cell immunosurveillance has been demonstrated in mice using inducible expression of the RAE-1 ligand by epithelial epidermis cells [89].

It was hypothesized that MICA was an activating ligand for Vδ1 T cells because the Vδ1 T cell distribution throughout the intestinal epithelium aligns with MICA expression [23, 90]. A functional relationship is proposed since Vδ1 T cell clones derived from colon carcinomas were able to specifically kill cells naturally expressing, or transfected with, MICA or MICB [91]. This recognition was inhibited by an anti-Vδ1 antibody, implicating the TCR in this process. Using MICA tetramers and Vδ1 TCR-transfectants, direct recognition of MICA by the Vδ1 TCR has been described [92]. This was confirmed by measuring the binding affinity of a single chain Vδ1 TCR to MICA of transfected Hela cells [93]. The affinity of the Vδ1 TCR for MICA was measured using surface plasmon resonance (Kd=3 μM). This affinity was close to that of NKG2D for MICA (Kd=1μM) [94]. These affinities where calculated by using soluble forms of the ligands and receptor and thus do not account for the real affinities and avidities of surface expressed molecules. The question of the functional relevance of the MICA/Vδ1 TCR interaction versus MICA/NKG2D on γδ T cells which constitutively express NKG2D is still open.

### 2. CD1

The second set of Vδ1 TCR ligands described was the CD1 molecules. These non-polymorphic MHC class I-like molecules have been well-characterized for presenting bacterial or synthetic lipid and glycolipid antigens [95, 96]. Humans have five CD1 proteins (CD1a, b, c, d and e) encoded by genes located on chromosome 1.

Two decades ago, several studies reported the recognition of CD1c by γδ T cells [97, 98], which formed the foundation for comparisons of γδ T cells and the multiple NKT cell populations. Ten years later, two Vδ1 T cell lines were derived in the presence of CD1-expressing DCs from blood of two healthy donors. These cells proliferated in response to DCs, and specifically killed CD1c transfected cells in a perforin and Fas-dependent manner [99]. Production of TNFα by CD1c-restricted Vδ1 T cells following interaction with DCs induces DC maturation [100]. The absence of foreign antigen in this model suggests that Vδ1 T cells can recognize self-antigens on antigen-presenting cells. This could provide the human immune system with the capacity to rapidly generate a pool of mature DCs early during infection, prior to antigen processing.

CD1c is not the only CD1 molecule able to activate Vδ1 T cells. Peripheral Vδ1 T cells from patients allergic to cypress proliferate in response to HeLa cells transfected with CD1a or CD1d and incubated with phospholipids extracted from cypress pollen [101]. Interestingly, some of the pollen extract-expanded Vδ1 T cell clones reacted to phospholipids not present in the pollen extract, but that are abundant in the human body, specifically in pulmonary surfactant. This observation supports the tendency towards recognition of self antigens by Vδ1 T cells. Vδ1 T cell clones isolated from normal duodenum can also be activated in the presence of CD1a-, CD1c- or CD1d-transfected cells and phospholipids [102]. CD1-reactive γδ T cell clones display distinct Vδ1 junctional regions and can use various Vγ gene segments. The proliferation of duodenum Vδ1 T cells in the presence of CD1-expressing cells is abrogated by anti-TCR γδ or anti-CD1 antibodies, suggesting TCR-mediated recognition [102]. Furthermore, the hypothesis that CD1c interacts with the Vδ1 TCR was supported by successful transmission of anti-CD1c reactivity through transfer of the cDNA encoding for one specific Vδ1-chain [99]. However, a direct examination for cognate interactions between CD1c and the Vδ1 TCR through surface plasmon resonance, for instance, has not been performed.

### 3. Unknown Ligands

A main issue which remains to be clarified regarding Vδ2$^{neg}$ γδ TCR ligands is the physiological frequency of CD1 or MICA responsive γδ T cells. Vδ1 TCR-mediated recognition of MICA and CD1 has been shown using several clones isolated from colon carcinomas and two peripheral blood T cell lines, and several clones isolated from normal duodenum, respectively. *Ex-vivo* staining of Vδ1 T cells with CD1 or MICA tetramers, as routinely done for CD8 αβ T cells with MHC-peptide tetramers or for T22/T10-specific murine γδ T cells [103], could provide information in this matter. Importantly, blocking antibodies against either MICA or CD1 do not abrogate the recognition of microbial-activated DCs or numerous tumor targets by Vδ2$^{neg}$ γδ T cells. Moreover, CMV-infected cells recognized by Vδ2$^{neg}$ γδ T cells do not express surface MICA, nor CD1 [86]. Therefore, it ensues that the cognate ligands for many Vδ2$^{neg}$ γδ TCRs remain unidentified.

## F. EVOLUTION OF EFFECTOR FUNCTIONS OF Vδ2$^{NEG}$ γδ T CELL DURING THE COURSE OF IMMUNE RESPONSES : EARLY EFFECTORS TO LATE REGULATORS

Even though the physiological and pathophysiological roles of Vδ2$^{neg}$ γδ T cells are not yet fully understood, several studies during the past decade provided interesting advances in the elucidation of Vδ2$^{neg}$ γδ T cell effector functions *in vivo*. Their role may turn out to be dependent on organ-, host- and disease-specific factors, as is true for αβ T cells. Furthermore, Vδ2$^{neg}$ γδ T cell functions evolve during the course of immune responses, from early effectors to late regulators.

### 1. Anti-Tumour Effectors

*In vitro*, Vδ2$^{neg}$ γδ T cells display typical CTL functions by killing their target cells through perforin/granzyme, or Fas/Fas-ligand pathways and by producing TNFα and IFNγ. Although informative, *in vitro* studies are inadequate to explore the actual involvement of γδ T cells in host anti-tumor responses. Murine xenograft models using immunodeficient mice are useful tools for testing the capacity of γδ T cells to effectively hamper or inhibit tumor growth, their migratory potential towards cancer cells, and their eventual contribution to limiting tumor spreading. Both Vδ2 and Vδ1 T cells expanded *ex vivo* from peripheral blood mononuclear cells (PBMCs) from melanoma patients could prevent the growth of autologous tumors when co-inoculated subcutaneously (s.c.) with cancer cells into SCID mice. However, when γδ T cells were infused intravenously (i.v.), only Vδ1 T cells could migrate towards s.c. implanted cancer cells and inhibit tumor growth [104].

We used a murine xenograft tumor model where HT29 cells were implanted under the skin of Rag$^{-/-}$γc$^{-/-}$ mice to evaluate the anti-tumor potential of a Vδ5 T cell clone reactive towards CMV-infected cells, as described above [63]. The solid HT29 tumors produced mainly pro-inflammatory chemokines, including MIP-1δ and MCP-4. When injected at a distance from the tumor site, Vδ5 T cell clones delayed HT29 tumor growth. The activated Vδ5 T cells expressed the receptor CCR3, and addition of an anti-CCR3 antibody abrogated this effect. These findings emphasize that CMV-induced Vδ2$^{neg}$ γδ T cells can exert an anti-tumor activity *in vivo*, and may be particularly relevant for transplant recipients who are at a higher risk for cancer [105].

The anti-tumor response of γδ T cells in humans is more difficult to analyze. Recently, it was found that γδ TILs are rare in renal cell carcinomas and that the percentage of γδ TIL did not correlate with any prognosis, including mortality rate [106]. However, the early effects of γδ T cells on emerging cancer cells cannot be investigated using late stage tumors. Interestingly, a significant positive correlation was found between Vδ1 T cell infiltration in melanomas and patient survival [17]. Vδ1 TILs were present in 52.7% of necrotizing melanomas and in only 14% of non-necrotizing melanomas. Furthermore, Lamb *et al.* have described a significant correlation was found between increased peripheral blood Vδ1 T cells in patients with acute lymphoblastic leukemia and long-term relapse-free survival, following bone marrow transplantation [78]. Vδ1 T cells were also involved in immune responses against chronic B-cell lymphocytic leukemia. No progression occurred in patients with increased circulating Vδ1 T cells in a 1-year follow-up, which was in contrast to patients with low numbers of peripheral blood Vδ1 T cells. Of note, low risk patients mostly displayed increased levels of Vδ1 T cells (100-300 cells/μl), as compared with most intermediate risk patients, all high-risk patients, and healthy donors (50-100 cells/μl). Interestingly, the high proportion of Vδ1 T cells in the blood was associated with the expression of the ULBP3 protein on autologous leukemic cells [81]. In another study, Catellani *et al*. identified an expansion of peripheral blood Vδ1 T cells producing IL4 in patients with low-grade non-Hodgkin B cell lymphomas. In most of these patients, B cells (from blood, lymph nodes or bone marrow) expressed the ULBP2 and/or ULBP3 proteins, which are associated with a lower rate of disease progression [80].

Finally, our recent work suggests that transplant recipients with increased numbers of circulating Vδ2$^{neg}$ T cells are less prone to develop cancer. We performed a longitudinal case control study in which γδ T cell levels of kidney transplant recipients were determined prior to the onset of cancer. γδ T cell levels of these patients were followed for 8 years to determine the risk factors of malignancy. The median γδ T cell percentage at 18, 12, and 6 months prior to cancer diagnosis was significantly lower than that of patients who did not develop cancer (manuscript submitted). This significant association between the increase of Vδ2$^{neg}$ γδ T cells and lower cancer occurrence was only observed in kidney transplant recipients who experienced pre- or post-graft CMV infection. In addition, in a separate cohort study kidney transplant recipients naive for CMV had a greater risk of cancer than patients who were exposed to CMV. These results highlight an unexpected protective role of CMV against cancer in kidney transplant recipients. This effect could be due to CMV-activated Vδ2$^{neg}$ γδ T cells that were cross-reactive towards tumor cells.

### 2. Anti-CMV Effectors: Adaptive Response?

In CMV-infected patients, the increase of Vδ2$^{neg}$ γδ T cells is positively correlated with the resolution of the viremia. Delayed expansion of Vδ2$^{neg}$ γδ T cells after the onset of viremia is associated with a longer and more intense infection and with more severe disease [107]. This suggests

an anti-viral function for γδ T cells. As demonstrated *in vitro*, Vδ2$^{neg}$ γδ T cells kill CMV-infected cells, limit CMV replication, and produce the anti-viral cytokine IFNγ. Since these cells are localized in epithelial tissues that are entry and/or replication sites for CMV (respiratory, digestive and genital mucosa), they may act very early in the anti-viral response, rapidly proliferate, and recirculate to patrol other infected sites (Fig. **2**).

Beside this early anti-viral role, Vδ2$^{neg}$ γδ T cells likely play a role later in the adaptive immune response to CMV, as well. Vδ2$^{neg}$ γδ T cells share many features with CMV-specific CD8 αβ T cells. A similar kinetics with long-term increase in percentage of both Vδ2$^{neg}$ γδ T cells and CMV-specific CD8 αβ T cells is observed in CMV-seropositive healthy individuals. Moreover, both cell subsets display the same phenotype: high levels of CD16, CD158, CD57, perforin and granzyme [66]. Longitudinal studies in transplant patients revealed a striking concomitant increase of Vδ2$^{neg}$ γδ T cell and CMV-specific CD8 αβ T cell percentages in peripheral blood during the course of CMV-infections [108]. The Vδ2$^{neg}$ γδ T cells and CMV-specific CD8 αβ T cells from these patients also display the same TEMRA phenotype. This peculiar phenotype of CD8 αβ T cells is unique to CMV infections among other persistent viral infections [109], and has also been observed for CMV-specific CD4 T lymphocytes [110]. Thus, it is reasonable to think that Vδ2$^{neg}$ γδ T cells might play a similar long-term protective function as do CMV-specific CD8 αβ T cells. However, further studies of organ recipients are still required to resolve this issue. Importantly, Vδ2$^{neg}$ γδ T cells expand more rapidly during a secondary response to CMV when compared to a primary infection in transplant patients, suggesting their adaptive flexibility [66]. Then Vδ2$^{neg}$ γδ T cells contribute to the pool of CMV-specific cells, which accumulate over time in chronically infected patients. This phenomenon, known as memory inflation, is observed both in human and murine CMV-infected individuals [111, 112]. Despite their TEMRA phenotype, CMV-specific αβ T cells have been shown to be able (i) to divide *in vitro* (with IL15), (ii) to respond to viral reactivation by expanding *in vivo,* and (iii) to provide protection *in vivo* [113-116]. TEMRA Vδ2$^{neg}$ γδ T cells could behave the same way and play an important long-term protective role in immunity to CMV.

### 3. Late Regulators

The function of Vδ2$^{neg}$ γδ T cells may change during the course of an immune response, or distinct subsets of Vδ2$^{neg}$ γδ T cells may exert different functions at various stages of a response. Vδ2$^{neg}$ γδ T cells possess features of both innate and adaptive immunity, and may play a role in bridging these two responses. Their ability to activate DCs through secretion of TNFα and IFNγ, or the expression of Fas-ligand is one example of such roles [72, 100]. After an initial cytolytic action against infected, stressed, damaged or transformed cell targets, data indicate that Vδ2$^{neg}$ γδ T cells may also perform critical regulatory functions, reminiscent of what have been documented for murine γδ T cells [117]. For example, Vδ1 T cell clones isolated from the synovial fluid of Lyme arthritis patients express high levels of Fas-ligand and are able to induce apoptosis of Fas-expressing synovial CD4 T cells [118]. The ability of Vδ1 TILs infiltrating breast tumors to suppress naïve and effector T cells as well as the maturation and function of DCs is another example [119].

Vδ1 T cell expansion has been associated with operational tolerance in liver allograft recipients whose immunosuppressive treatment has been withdrawn [120-122]. Since Vδ1 T cells expand upon CMV challenge in these patients, and since CMV has immunosuppressive functions, one cannot rule out a role of the virus in this association. Vδ1 T cells have been also incriminated in pregnancy-associated tolerance. In peripheral blood of healthy pregnant women, the most frequently occurring γδ T cell population expresses the Vγ4Vδ1 TCR, whereas in women with recurrent miscarriages, the Vγ9Vδ2 population is predominant [123].

In allergic patients, CD1/phospholipid-restricted Vδ1 T cells were shown to produce IFNγ, but also IL4 and TGFβ, upon phospholipids stimulation. From these data, it is difficult to extrapolate their putative role *in vivo*. Vδ1 T cells could play a regulatory role to protect the host from harmful inhaled products or damaging hypersensitivity through TGFβ production. Alternatively, they could also participate in inflammatory or allergic responses. Notably, their Th2-like activity of inducing IgE production might favor a role in the pathogenesis of allergic disease [124]. Because CD1 molecules are widely expressed by epithelial cells and DCs in mucosal tissues, γδT cells can function as a first line of defense and shift the response towards Th1 versus Th2, or regulation versus inflammation.

## G. A PLACE FOR Vδ2$^{NEG}$ γδ T CELL MANIPULATION IN NEW THERAPEUTIC PROTOCOLS

Important advances have been made in the understanding of the functions of human γδ T cell subsets, which now allow exploring their potential utility in immunotherapy protocols. The diversity of their immunological functions and their lack of known MHC-restriction are of particular interest. Stimulation of Vγ9Vδ2 T cells with phosphoantigens or aminobiphosphonates are ongoing strategies already used in phase I and II anti-tumor clinical trials [125]. The functions of Vδ2$^{neg}$ γδ T cells in immune responses to very diverse pathological settings as recapitulated above make them attractive candidates for immunotherapy, for both cancer and infectious diseases. Future research efforts will be focused on identification of the activating ligands recognized by this subset in the different physiopathological contexts. Once this goal will be completed, the immune response could be enhanced in two ways, either *via in vivo* administration of the ligands to stimulate Vδ2$^{neg}$ γδ T cells *in situ*, or *via ex vivo* activation of Vδ2$^{neg}$ γδ T cells with the ligands for adoptive transfer protocols.

## ACKNOWLEDGEMENTS

We are grateful to Jean-François Moreau for critical reading of the manuscript. We thank ANR, FRM, ARC, LNCC, ABM and DGA for support.

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Received: June 22, 2009 Revised: July 2, 2009 Accepted: July 8, 2009