

# Quantitative Peripheral Blood Perturbations of $\gamma\delta$ T Cells in Human Disease and Their Clinical Implications

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**Abstract** Human  $\gamma\delta$  T cells, which play innate and adaptive, protective as well as destructive, roles in the immune response, were discovered in 1986, but the clinical significance of alterations of the levels of these cells in the peripheral blood in human diseases has not been comprehensively reviewed. Here, we review patterns of easily measurable changes of this subset of T cells in peripheral blood from relevant publications in PubMed and their correlations with specific disease categories, specific diagnoses within disease categories, and prognostic outcomes. These collective data suggest that enumeration of  $\gamma\delta$  T cells and their subsets in the peripheral blood of patients could be a useful tool to evaluate diagnosis and prognosis in the clinical setting.

**Keywords** Gammadelta T cells · Gamma delta T cells · Human diseases · Differential diagnosis · Prognosis · Peripheral blood gammadelta T cells

## Introduction

Intensive research in the 1980s led to the discovery of the rearranging  $\alpha$  and  $\beta$  genes encoding the  $\alpha\beta$  T cell receptor (TCR), which is expressed on CD4+ and CD8+ T cells [1–4]. However, at that time, the role of a third, rearranging gene,  $\gamma$ , initially detected in a clone of cytotoxic T cells expressing the  $\alpha\beta$  TCR, remained a mystery [5]. The enigma of the  $\gamma$  gene was resolved when lymphocytes bearing  $\gamma\delta$  TCR, were unveiled in 1986 [6, 7]. In those studies, clones of CD4– and CD8– cytotoxic and cytokine producing thymocytes and

peripheral blood (PB) T cells expressing a CD3-associated heterodimeric TCR encoded by the  $\gamma$  gene, along with a novel polypeptide encoded by an additional rearranging gene,  $\delta$  were derived from human thymus and the PB of patients with immunodeficiencies [6, 7]. Although subsequent studies revealed major functional overlap between T cells expressing either  $\gamma\delta$  or  $\alpha\beta$  TCR ( $\gamma\delta$  or  $\alpha\beta$  T cells, respectively), modes of antigen recognition and activation of each subset are distinct. The most striking difference between the two subsets is the lack of requirement of antigen processing and presentation by major histocompatibility (MHC) molecules for  $\gamma\delta$  T cells, allowing more rapid response to their respective antigens [8]. Moreover, the antigenic repertoire of  $\gamma\delta$  T cells appears to be quite unique. Most striking, and as opposed to  $\alpha\beta$  T cells whose TCR are designed to recognize peptides presented by autologous MHC, an entire human  $\gamma\delta$  T cell subset, expressing a TCR heterodimer encoded by V (variable)  $\gamma 9V\delta 2$  genetic combinations, is primed during infancy and early adulthood to respond to phosphorylated metabolic intermediates of bacteria and protozoa [9]. These cells also recognize (with lower affinity) corresponding intermediates of the mevalonate pathway (e.g., isopentenyl pyrophosphate, IPP) in eukaryotic cells that increase during cellular stress induced by infection, cancer, and inflammation [10]. Although the mechanism of recognition of the phosphorylated moieties by the  $V\gamma 9V\delta 2$  TCR remains a mystery, recent data suggest involvement of butyrophillin CD277 as a molecule involved in sensing perturbations of IPP in target cells, which mediates  $V\gamma 9V\delta 2$  activation [11].

Importantly, exposure of  $V\gamma 9V\delta 2$  T cells to cells (e.g., monocytes, fibroblasts, tumor cells) expressing high levels of these non-peptidic antigens, initiates a cascade of clinically relevant immune responses including cytokine release, activation of other T cells, dendritic cells and B cells as well as potent cytotoxicity [9]. The second major human subset of  $\gamma\delta$  T cells expresses the  $V\delta 1$  gene, is located for the most part at

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mucosal surfaces, in particular within the lining epithelium as a significant proportion of the “intraepithelial T cell compartment” and appears to have specialized to recognize lipids presented by cluster of differentiation (CD) 1d [12]. V $\delta$ 1+ T cells, which for the most part are also CD4– and CD8– may also express CD8 $\alpha\alpha$ , are potent producers of cytokines and also mediate cytotoxicity against malignantly transformed cells. Table 1 summarizes major features of human  $\gamma\delta$  T cells in comparison with  $\alpha\beta$  T cells.

Excellent reviews of the basic biology of  $\gamma\delta$  T cells have been published highlighting their unique role in the murine and human immune systems [13–15]. Although many insights have been gleaned from studies of murine  $\gamma\delta$  T cells, including the mode of antigen recognition by the TCR, processes of differentiation in the thymus, and the emergence of subsets during intrathymic differentiation, striking differences between murine and human  $\gamma\delta$  T cells are evident. For example, an entire subset of murine  $\gamma\delta$  T cells known as dendritic T cells (DETC), all expressing a single TCR, densely populates the murine epidermis, but an equivalent population is not present in human skin. Another intriguing difference is that in murine systems, recent data have clearly differentiated a population of  $\gamma\delta$  T cells that are primed during differentiation to produce interleukin (IL) -17, a cytokine that plays a crucial role in inflammatory responses, and these cells continue to secrete this cytokine in the periphery [16]. Although human  $\gamma\delta$  T cells also can produce IL-17 after stimulation, this function has not as yet been linked to a clearly defined subset, but may be context dependent in the periphery. Thus, for example, activation of V $\gamma$ 9V $\delta$ 2+ cells with IPP in the presence of IL-1 $\beta$ , IL-23, IL-6, and TGF $\beta$  and aromatic hydrocarbons, will result in the induction of secretion of IL-17 in cells with a CD45RA+CCR6+CD161+TRAIL+FasL+ phenotype [17]. Conversely, there is no equivalent of the human phosphoantigen-reactive V $\gamma$ 9V $\delta$ 2 T cells in mice. Thus, the role of  $\gamma\delta$  T cells in murine models of immune regulation and disease may not appropriately simulate the corresponding human situation. On the other hand, primates, whose immune system is similar to humans and includes a phosphoantigen-reactive subset, are much less amenable to extensive experimentation [18]. These considerations imply that in order to understand more fully the role of the unique  $\gamma\delta$  T cell subset in humans, direct evaluation of these cells in human health and disease are crucial.

Indeed, the unique roles and significance of  $\gamma\delta$  T cells during human clinical disease are gradually being uncovered. In particular, perturbations of numbers and phenotype of  $\gamma\delta$  T cells in PB and in tissues have been observed in cancer, infections, allergy, and autoimmunity, and individual studies suggest that these alterations can be related to specific diseases and carry prognostic implications. However, enumeration of  $\gamma\delta$  T cells in PB has not as yet entered routine clinical practice, which hampers further progress in understanding how these crucial immune cells participate in human health.

**Table 1** Characteristics of human  $\gamma\delta$  compared to  $\alpha\beta$  T cells

	$\gamma\delta$ T cells		$\alpha\beta$ T cells	
	V $\delta$ 1	V $\gamma$ 9 $\delta$ 2	CD4	CD8
Percent among peripheral blood CD3+ T cells	0.5–2	2–5	~70	~25
CD4 expression	No	No–rare	Yes	No
CD8 expression	Some	No–rare	No	Yes
Response to peptide antigens	Yes?	No	Yes	Yes
Respond to lipid antigens	Yes	No	Rare	No
Recognition of low molecular weight phosphorylated metabolites	No	Yes	No	No
Direct activation by amino - bisphosphonates	No	Yes	No	No
Antigen presented by MHC	No	No	Yes	Yes
TNF $\alpha$	Yes	Yes	Some	Yes
T1 cytokines	Yes	Yes	Yes	Yes
T2 cytokines	Rare	Rare	Yes	Yes
T17 cytokines	Yes	Yes	Yes	Yes
Regulatory function	Yes	Yes	Yes	Yes
Cytotoxic function	Yes	Yes	Weak	Yes
B cell helper function	Yes?	Yes	Yes	No
Mucosal intraepithelial location	Yes	No	No	Yes

Functional characteristics of the indicated T cell subsets. T1, T2, and T17 cytokines refer to T cells producing IFN $\gamma$  (T1), IL-4 and IL-13 (T2), and IL-17(T17)

To evaluate whether numerically measurable perturbations of PB  $\gamma\delta$  T cells and their subsets could indeed serve as a useful clinical tool, we reviewed studies available in the PubMed database reporting numerical evaluations and alterations of these cells in human diseases, with the exclusion of primary immunodeficiency syndromes and malignant conditions of the  $\gamma\delta$  T cells themselves.

In the following sections, we detail alterations in levels of PB  $\gamma\delta$  T cells in physiological conditions and within specific diseases. The combined results of this review suggest that enumeration of PB  $\gamma\delta$  T cells could be an important tool in routine clinical practice in a variety of conditions. Importantly, because many of the studies report analysis of both total numbers and percentages of these cells, as well as subsets within the total population of  $\gamma\delta$  T cells, we herein provide a brief review of how human  $\gamma\delta$  T cells are divided into further subsets, and the significance of each.

### Human $\gamma\delta$ T Cell Subsets

The primary division within the human  $\gamma\delta$  T cell subsets is based on the expression of the V region genes of the  $\gamma$  and  $\delta$  TCR chains. Cells expressing V $\gamma$ 9 and V $\delta$ 2 (V $\gamma$ 9V $\delta$ 2 T cells) are present mainly in PB and lymphoid organs. In contrast, those expressing V $\delta$ 1 with a gene other than V $\gamma$ 9 within the

**Table 2** Subsets of human V $\gamma$ 9V $\delta$ 2  $\gamma\delta$  T cells

Subset	Cell surface markers (percent within all V $\gamma$ 9V $\delta$ 2)	Proliferation to IPP+IL-2	Cytokine receptors	Cytokines	Receptors associated with cytotoxic function	Cytotoxicity
Naïve ( $T_{NAIVE}$ )	CD27+CD45RA+CCR7+CD62L+ CCR2, 5,6-CXCR3– (10–20 %)	+		–	NKG2D	–
Central memory (TCM)	CD27+CD45RACD45RO+, CCR7+ CD62L+CCR2, 5,6-CXCR3– (25–50 % in PB, 50 % in lymph nodes)	+	CXCR5 (15 %)	Low levels	NKG2D	Weak
Effector memory (TEM)	CD27–CD45RA–CD45RO+, CCR7 –CD62LCCR2, 5,6+CXCR3+ (30 %)	Lower than TCM	CCR5	High levels of IFN $\gamma$ and TNF $\alpha$	NKG2D	Weak
Terminally differentiated effector memory (TEMRA)	CD27–CD45RA+, CCR7–CD62L–CCR5+, CXCR3+, CD16+, KIR2DL1–3+ NKG2A/CD94+ (<7 % in PB, abundant in tissues)	Weak or absent		Low	NKG2D, NKG2C, CD56, CD16 KLRG1	Strong
IL-17 producers	CD27–CD45RA+CD161+CCR6+	Require IL-1 $\beta$ , IL-6, TGF $\beta$ , IL-23, aromatic hydrocarbons		IL-17A	NKG2D	–

Attributes of each of the human V $\gamma$ 9V $\delta$ 2  $\gamma\delta$  T cell subsets on the left

CCR and CXCR chemokine receptors; NK natural killer; IL interleukin; TGF transformin growth factor; KLR killer cell lectin-like receptor; NKG natural killer group; KIRDL1 killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 1; PB peripheral blood

TCR $\gamma$  polypeptide are more prevalent in tissues. Major characteristics of each of these subsets, in comparison to the major subsets of  $\alpha\beta$  T cells are summarized in Table 1. A second fundamental classification pertains to the status of the cells with respect to previous antigen stimulation and acquisition of a memory or effector functional status. Most commonly this classification is applied to the V $\gamma$ 9V $\delta$ 2 subset, for which antigens (IPP and (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate, HMBPP) have been identified, and is based on the expression of surface membrane markers CD45RO, CD45RA, and CD27. Thus, CD27+CD45RA+CD45RO– are thought to represent naïve cells, a CD27+CD45RO+CD45RA– phenotype is a hallmark of a “central memory” cell (TCM), whereas CD27–CD45RA– cells are effector memory  $\gamma\delta$  T cells (TEM). A further subset is CD27–CD45RA+ and known as TEMRA, i.e., effector memory terminally differentiated cells that express potent cytotoxic functions. Other markers associated with subsets of  $\gamma\delta$  T cells are chemokine and cytokine receptors, and cytokines secreted by specific subsets. The functions of each subset are designated in Table 2, which is based on data presented in reviews by Pang et al. and Kabeliz [17, 19].

### Association of $\gamma\delta$ T Cell Alterations in Peripheral Blood with Physiological Conditions in Humans

**Age** Age is associated with numerical alterations of  $\gamma\delta$  T cells and their subsets. V $\delta$ 1 cells predominate during fetal and early

life in the PB of humans. In most studies, these represent the minority in the PB of adults, whereas V $\delta$ 2 cells, which exhibit features of cells previously activated by antigens (“memory” T cells) constitute the majority of adult.

PB  $\gamma\delta$  T Cells [20]. O’Leary first noted that elderly (>69 years old) patients among clinic subjects with chronic disease (e.g., osteoarthritis or congestive heart failure) had a consistent decrease in the percent of CD3+ lymphocytes due to a 5–10 % reduction of CD3+, TCR $\alpha\beta$ -negative (i.e.,  $\gamma\delta$  T cell receptor bearing) T cells in the peripheral blood mononuclear cell (PBMC) population compared to young adults [21]. This absolute number of circulating  $\gamma\delta$  T cells was shown to be reduced significantly in old people and centenarians in comparison with young subjects as a result of an age-dependent reduction of V $\delta$ 2 T cells, whereas the absolute number of V $\delta$ 1 T cells was unaffected by age. The decreased absolute number of the V $\gamma$ 9V $\delta$ 2 T cells was due to the reduction of naïve (antigen inexperienced) and “central” memory (antigen experienced) cells capable of circulating in the lymphatic system) V $\gamma$ 9V $\delta$ 2 T cells bearing the CD27 and CCR7 cell surface molecules [22]. A comprehensive study confirmed that V $\gamma$ 9V $\delta$ 2 T cell numbers change characteristically with age, rising from birth to puberty and gradually decreasing again beyond 30 years of age. In adults, female blood donors have significantly higher levels than males, implying that circulating V $\gamma$ 9 $\delta$ 2 T cells in women remain elevated for a longer period in life. In this study, the loss (in men) was associated with depletion of effector memory CD27– CD45RA– and CD27– CD45RA+ T cells and a

parallel increase in CD27+ CD45RA– central memory T cells, while in women, the distribution of V $\gamma$ 9V $\delta$ 2 T cell subsets remained virtually unchanged [23]. Similar findings were reported in 120 healthy Japanese individuals in whom the average numbers of T lymphocytes in a cubic millimeter of blood were 1,084 $\pm$ 369 (SD)  $\alpha\beta$  T cells, 68 $\pm$ 44  $\gamma\delta$  T cells, 16 $\pm$ 12 V $\delta$ 1 T cells, and 43 $\pm$ 36 V $\delta$ 2 T cells and the absolute numbers of  $\gamma\delta$  T cells decreased with aging ( $R=-0.378$ ,  $P<0.001$ ) as a result of reduction of V $\delta$ 2, but not of V $\delta$ 1, T cells. However, in this study, numbers of V $\delta$ 2 T cells were significantly higher in male than in female donors [24]. Interestingly, the decrease of total  $\gamma\delta$  T cells with age (4 % in young vs 2 % in the old) is also influenced by cytomegalovirus infection status, being limited to uninfected individuals, whereas the decrease of V $\delta$ 2 T cells specifically, occurs in both cytomegalovirus (CMV)+ and in CMV– individuals (from 4 to 0.8), along with an increase in V $\delta$ 2–  $\gamma\delta$  T cells (0.9 to 2) only in CMV+ individuals (Fig. 1) [25].  $\gamma\delta$  T cells from old people and centenarians show enhanced levels of the early cell surface membrane activation marker CD69 both after culture in medium alone and in lipopolysaccharide (LPS), a basal increased production of TNF $\alpha$  and an impaired response to IPP [26]. In another study, a higher percentage of  $\gamma\delta$  T cells producing TNF $\alpha$ , but not IFN $\gamma$ , was found in old donors and centenarians [27].

**Race and Origin** The frequency of PB  $\gamma\delta$  T cells in healthy West Africans was reported to be about twice that of Caucasians, but mainly due to a fivefold increase in V $\delta$ 1+ cells, which is consequently the dominant subset in West Africans (Fig. 1b) [28]. Indeed, in adult European donors the major  $\gamma\delta$  T cell population in PB is V $\gamma$ 9+ (approximately 70 % of all  $\gamma\delta$  cells), whereas in the majority of adult Africans V $\gamma$ 9– V $\delta$ 1+ T cells predominated (approximately 70 % of total  $\gamma\delta$  cells) [29]. Likewise, substantial differences in the V $\gamma$ 9 repertoire for cord blood collected in Jos (Nigeria, a malaria endemic endemic) or Rome

were noted, which were consistent with a negative selection mechanism operating on the fetal V $\gamma$ 9 chain repertoire in neonates from Jos, perhaps due to exposure to malaria, as discussed below [30]. In the USA, Caucasian donors had 3.71 $\pm$ 4.37 % V $\delta$ 2 cells (as a percentage of total lymphocytes in PB) compared with 1.18 $\pm$ 2.14 % V $\delta$ 2 cells for African American donors ( $P<0.0001$ ). In addition, age as well as race had the greatest impact on V $\delta$ 2 cell levels, and the effect of age was similar for both racial groups [31] (Fig. 1b). Finally, in Sweden, the percentage of  $\gamma\delta$  T cells was higher in Bangladeshi than Swedish subjects [32]. Thus, genetic as well as environmental influences are clearly important in determining the composition and numbers of  $\gamma\delta$  T cells in human PB.

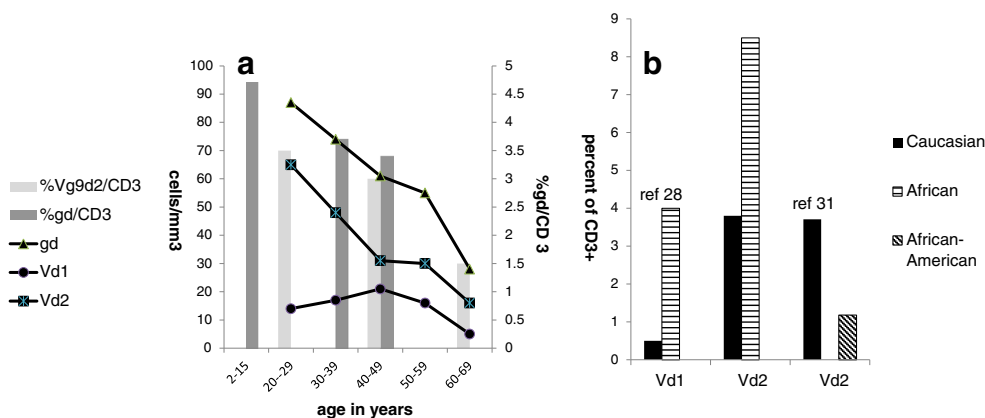
**Pregnancy** In PB of healthy pregnant women the percentage of  $\gamma\delta$  TCR+ cells was significantly higher than in recurrent aborters or non-pregnant individuals. Interestingly, 97 % of  $\gamma\delta$  TCR+ pregnancy lymphocytes expressed progesterone receptor [33].

## Alterations of $\gamma\delta$ T cells in Diseases:

### Infectious Disease

#### Human Acquired Immunodeficiency Syndrome (HIV)

Expansion of V $\delta$ 1 T cells occurs in both HIV-1 and HIV-2 infections but is accompanied by a significant contraction of the V $\delta$ 2 T cell population only in the former [34] (Fig. 2). Nevertheless, V $\delta$ 2 T cell activation was increased in both HIV-1– and HIV-2 and did not revert after treatment with highly active antiretroviral therapy (HAART) [34, 35]. Likewise, the high prevalence of memory (CD45RO+) and HLA-DR expressing V $\delta$ 1+ T cells in HIV-1-infected patients, but



**Fig. 1** Variation of peripheral blood (PB)  $\gamma\delta$  T cells with age (a) and race (b). a Mean of percent of indicated  $\gamma\delta$  T cell subsets of among CD3+ T cells (bars) or absolute numbers of cells per cubic millimeter of PB (lines) are shown in relation to age groups indicated on abscisse. Data

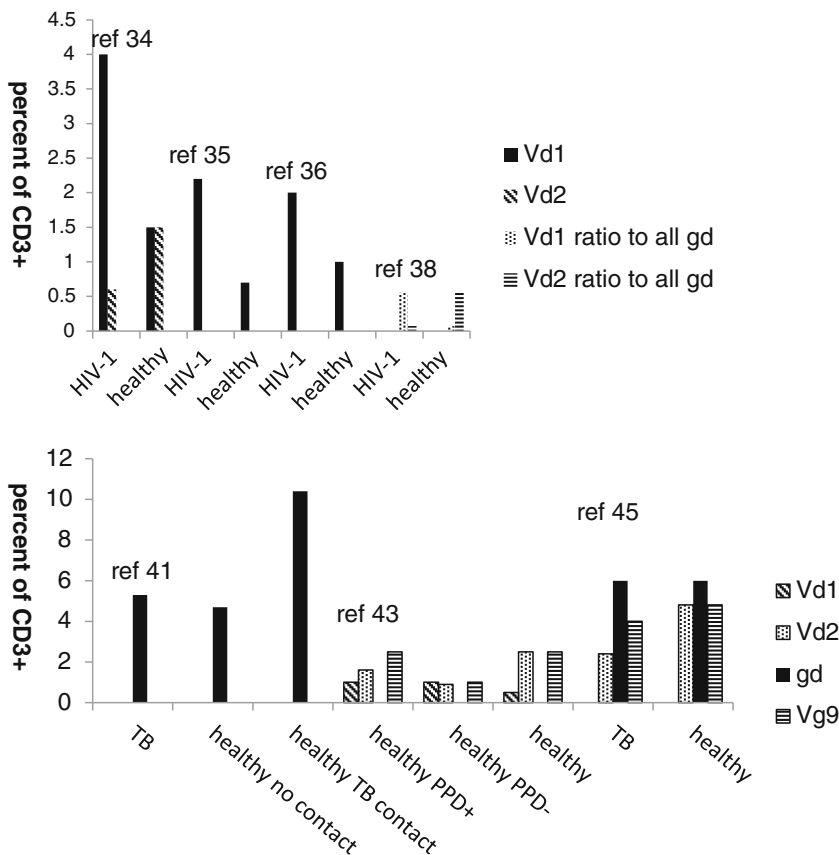
is based on ref. [22–24]. b Mean of percent among CD3+ T cells of indicated  $\gamma\delta$  T cell subsets. Bars below and to the right of indicated references (ref) represent values quoted from the referenced studies

not in healthy donors, indicates antigenic activation of these cells in HIV-1 [36]. Furthermore, compared with healthy HIV-negative subjects, HIV+ patients had a total decreased number of IFN $\gamma$ +  $\gamma\delta$  T cells due to a decrease of the number of V $\gamma$ 9V $\delta$ 2 cells, whereas the number of IFN- $\gamma$ +V $\delta$ 1+ cells was actually increased [37]. Indeed, at variance with healthy donors, in HIV-1-infected patients, ex vivo-isolated V $\delta$ 1 T cells co-express cytoplasmic proinflammatory cytokines IFN $\gamma$  and IL-17, as well as CD27, the surface marker of the central memory T cell subset [38]. In another study, HIV-1 infection was associated with significant expansion of V $\delta$ 1 and contraction of V $\delta$ 2 cell populations in mucosa in addition to PB during both acute and chronic HIV-1 [35]. Furthermore, the  $\gamma\delta$ -intraepithelial lymphocyte (IEL) ratio (median 14.5 %, range 1.5–56.3 %) was significantly increased compared with clinically healthy HIV (-) control subjects ( $n=11$ , median 2.8 %; range 0.3–38 %) [39]. Thus HIV is associated with activation of  $\gamma\delta$  T cells, with enhancement and activation of the V $\delta$ 1 and depletion of the V $\delta$ 2 subsets. Interestingly however, in HIV patients naive to antiretroviral therapy, combined treatment with intravenous zoledronate (zol) and sub-cutaneous IL-2, induced in vivo expansion and maturation of their V $\delta$ 2 T cells. Paralleling V $\delta$ 2 T cell activation, increased dendritic cell maturation and HIV-specific CD8 T cell responses were found [40].

*Tuberculosis (TB)*

Among health care workers who were tuberculin skin test positive and had constant contact with patients with active TB (“healthy contacts”) the percent of  $\gamma\delta$  T cells in fresh PB was significantly higher than in healthy noncontacts (Fig. 2). By contrast, patients with active pulmonary tuberculosis had low levels of  $\gamma\delta$  T cells [41]. The general decrease of  $\gamma\delta$  T cells, however, is accompanied by expansion of central memory V $\gamma$ 9V $\delta$ 2 T cells and reduction of the pool of effector memory (CD45RO+) and terminally differentiated cells (CD45RA+), which reversed after anti-mycobacterial therapy [42]. Further analysis in active pulmonary tuberculosis showed, moreover, that the size of the mycobacteria-reactive V $\gamma$ 9+V $\delta$ 2+ $\gamma\delta$  T cell subset in both the blood and lung was dramatically reduced compared with both normal healthy subjects and patients with unrelated pulmonary granulomatous diseases—sarcoidosis and berylliosis. In addition, the V $\gamma$ 9+V $\delta$ 2+cells left intact in patients with tuberculosis were refractory to in vitro stimulation by mycobacteria tuberculosis antigens which are potent stimuli for these cells in normal subjects [43] [44] [45] (Fig. 2). In another study of 27 patients with active pulmonary tuberculosis and 16 healthy donors (HD), the proportion of total IL-17-producing cells among lymphocyte was similar between TB patients and HD, but the

**Fig. 2** Peripheral blood (PB)  $\gamma\delta$  T cell subsets in human immunodeficiency virus-1 (HIV-1) and tuberculosis (TB). Mean percent of indicated  $\gamma\delta$  T cell subsets among CD3+ T cells, based on references indicated above and to the left of each set of values, are shown. Clinical status of studied groups is described along x-axis. ref, reference



proportions of  $\gamma\delta$  T cells in IL-17-producing cells and of IL-17-producing cells in  $\gamma\delta$  T cells in PB were markedly increased in TB patients (43.9 and 7.7 %, respectively) contrasting with lower levels of IFN $\gamma$ -producing  $\gamma\delta$  T cells [46].  $\gamma\delta$  T cells were also studied in tuberculosis-infected 24 HIV patients—11 with and 13 without immune reconstitution syndrome (IRS) that sometimes occurs after institution of highly active anti-retroviral therapy (HAART). At baseline, lower proportions of  $\gamma\delta$  T cells and V $\delta$ 2+ T cells and of those displaying the inhibitory receptors CD94/NKG2 and CD158ah,b (Killer inhibitory receptors, KIR) were found in the patients who developed IRS. During IRS, V $\delta$ 2+ T cells, mostly KIR $^{-}$ , significantly peaked as opposed to non-IRS patients suggesting that KIR $(-)$ V $\delta$ 2+  $\gamma\delta$  T cells play an immunoregulatory role in HIV patients developing IRS [47].

### Parasitic Infections

**Malaria** In Ethiopia,  $\gamma\delta$ + T cells were significantly elevated in patients infected with malaria falciparum (f) and in those doubly infected with f. malaria and vivax malaria but not in vivax malaria alone, compared to healthy controls, and the increase was mostly due to V $\delta$ 1+ cells (Fig. 3)[48]. Likewise, in Ghanaian children, both frequency and absolute number of  $\gamma\delta$  T cells, again mostly highly activated and polyclonal V $\delta$ 1 cells, were transiently increased to 30 to 50 % of all T cells shortly after initiation of antimalarial chemotherapy for plasmodium falciparum malaria [49] (Fig. 3). Furthermore, among nine non-immune patients who contracted malaria during travel to endemic areas (five with *Plasmodium falciparum* and four with *Plasmodium vivax* infections)  $\gamma\delta$  T lymphocytes expanded to comprise  $17.92\pm 11$  % of the PBMC (vs  $3.08\pm 2.4$  %  $\gamma\delta$  cells in normal control subjects). In these patients, V $\delta$ 2+ cells predominated, although cells expressing V $\delta$ 1 also

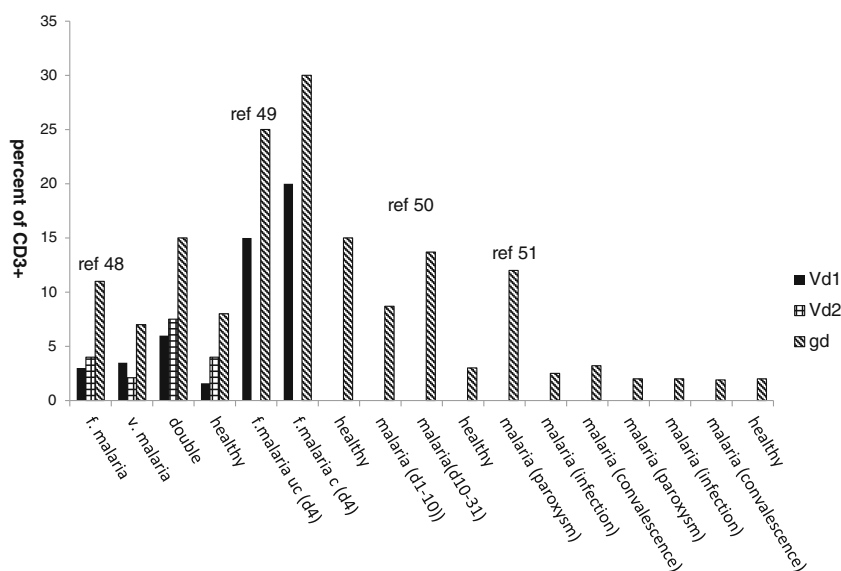
expanded significantly in some patients. The mean level of  $\gamma\delta$  cells peaked during the second month after the acute clinical syndrome, when patients were free of symptoms [50] (Fig. 3). In another study of non-immune patients, a highly significant increase in both the proportion and the absolute numbers of  $\gamma\delta$  T cells up to 30 % of PBMC, was observed in *Plasmodium vivax*-infected patients during clinical paroxysms compared to nonmalarial controls. Curiously, in an age-matched group of semi-immune patients resident in a malaria-endemic region, in whom the clinical disease was comparatively mild, there was no increase in  $\gamma\delta$  T cells during infection during paroxysms or convalescence [51] (Fig. 3). The potential strong effect of malaria exposure on  $\gamma\delta$  T cell subsets is highlighted by the finding of a substantial difference in the V $\gamma$ 9 repertoire for cord blood collected in Nigeria, a malaria endemic country and a non-endemic region (Rome, Italy) consistent with a negative selection mechanism (perhaps exposure to malaria) operating on the fetal V $\gamma$ 9 chain repertoire in Nigerian neonates [30].

**Other Parasites** Among 20 patients with *local cutaneous leishmaniasis*, significantly more  $\gamma\delta$  T cells were observed in untreated patients ( $15.9\pm 5.9$  %), when compared with glucantime-treated patients ( $4.6\pm 1.4$  %) and controls ( $5.3\pm 2.3$  %)[52]. In addition, pregnant women with *primary toxoplasmosis* displayed a significant decrease of the CD4/CD8 T cell ratio concurrent with a significant increase of circulating  $\gamma\delta$  T cells compared to uninfected counterparts [53].

### Viral Diseases Other than HIV

**Cytomegalovirus (CMV), Epstein Barr Virus (EBV), and Human Herpes Virus (HHV)-8 Infections** During the first 24 months after stem cell transplantation, significant long-term expansions of V $\delta$ 2 negative (–) but not V $\delta$ 2+  $\gamma\delta$  T cells was noted

**Fig. 3** Variation of  $\gamma\delta$  T cells in malaria. Mean percent of indicated  $\gamma\delta$  T cell subsets among peripheral blood CD3+ T cells, based on references (ref) indicated above and to the left of each set of values, are shown. Clinical status of studied groups is described along the x-axis. f, falciparum; v, vivax; double, vivax plus falciparum; d, day after infection



in patients with CMV reactivation early after transplantation. Similarly, significantly higher numbers of V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells were detected in CMV-seropositive healthy persons compared with seronegative donors. In contrast V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cell expansion was absent in CMV-negative/Epstein–Barr virus-positive patients [54] (Fig. 4). Effector memory  $\gamma\delta$  T cells likewise showed a concomitant increase and persistence in most kidney transplant recipients with CMV infection [55] (Fig. 4). Furthermore, a dramatic expansion of activated  $\gamma\delta$  T cells in the PB of renal allograft recipients developing CMV infection lasted more than 1 year, and involved only V $\delta$ 1 or V $\delta$ 3<sup>+</sup> T cells [56]. A comprehensive study of circulating  $\gamma\delta$  T cells in renal allograft recipients, using multivariate analysis confirmed that CMV infection was the only independent parameter associated with >6 %  $\gamma\delta$  T cells [57] (Fig. 4). Importantly, patients who developed a delayed  $\gamma\delta$  T cell expansion after CMV infection ( $\geq$ 45 days) had significantly longer and higher pp65 antigenemia and more-symptomatic CMV disease than did patients with early expansion. Furthermore,  $\gamma\delta$  T cell expansion was concomitant with the resolution of CMV infection [58] (Fig. 4). Fetal V $\gamma$ 9- $\gamma\delta$  T cells also specifically expand and become differentiated upon CMV infection in utero [59]. At a functional level, V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cell PB numbers, repertoire restriction, and cytotoxicity against CMV-infected fibroblasts were markedly increased in CMV seropositive, compared with CMV seronegative, healthy persons and exhibited a cytotoxic effector/memory phenotype only in the CMV<sup>+</sup> patients [60]. Together, these data strongly support an important role of V $\delta$ 2<sup>-</sup> in response to and resolution of CMV. In other studies, protracted (up to 5 months) elevated  $\gamma\delta$  T cell component occurs in Epstein Barr Virus (EBV) related but not EBV negative, infectious mononucleosis. HHV-8 infection has also been associated with significant expansion of effector  $\gamma\delta$  V $\delta$ 1<sup>+</sup> T cells, suggesting an important role of  $\gamma\delta$  T cells in herpes virus infection in general [61, 62] [63].

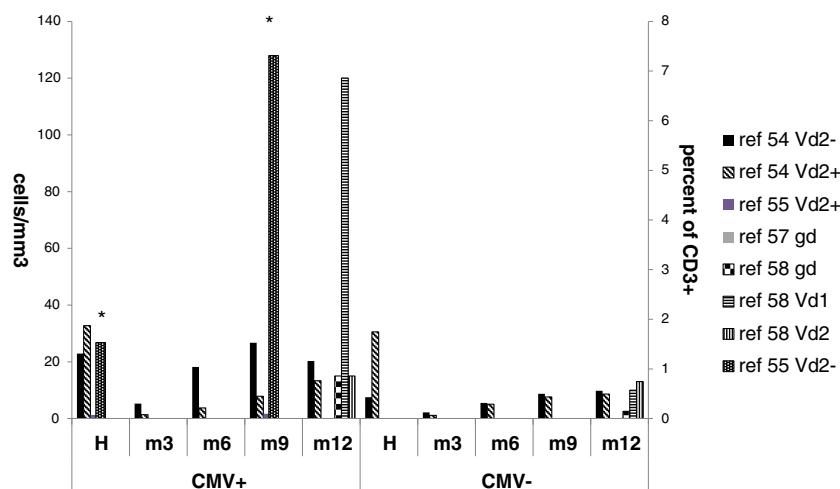
**Other Viral Infections**  $\gamma\delta$  T cells from *respiratory syncytial virus-infected* infants included a lower proportion of IFN $\gamma$ <sup>-</sup> (median, 4.00 %; range, 0.58–6.60 %) and a slightly higher proportion of IL-4-producing cells (median, 0.40 %; range, 0.13–2.76 %) than rotavirus-infected infants (median, 32.10 %; range, 14.43–61.21 %;  $P < 0.01$ , median [64]. In children with *dengue hemorrhagic fever* absolute  $\gamma\delta$  T cell counts were decreased compared with those with dengue fever early in the course of illness [65].

#### Bacterial Infections Other than Tuberculosis

**Salmonella** Proportions of  $\gamma\delta$  T cells in *Salmonella* infections were significantly increased ( $17.9 \pm 13.2$  %) compared with normal controls ( $5.0 \pm 2.6$  %) and patients with other bacterial infections ( $4.0 \pm 1.4$  %). Furthermore, the most prominent expansions occurred in the systemic ( $28.9 \pm 10.8$  %) rather than in the gastroenteritic form ( $10.5 \pm 7.9$  %) of salmonellosis. Furthermore,  $\gamma\delta$  T cell activation was significantly greater than  $\alpha\beta$  T cell activation during early illness (Fig. 5) [66]. In addition, the proportion of  $\gamma\delta$  T cells expressing NKR1A as well as IFN $\gamma$ +NKR1A+ $\gamma\delta$  T cells were significantly higher in *Salmonella* patients than in other intestinal infections or in healthy individuals [67]. Indeed, patients with *Shigella dysenteriae* and *Shigella flexnerii* revealed an expansion of  $\gamma\delta$  T cells in the rectal mucosa, but a decrease in blood compared to healthy age-matched controls [68].

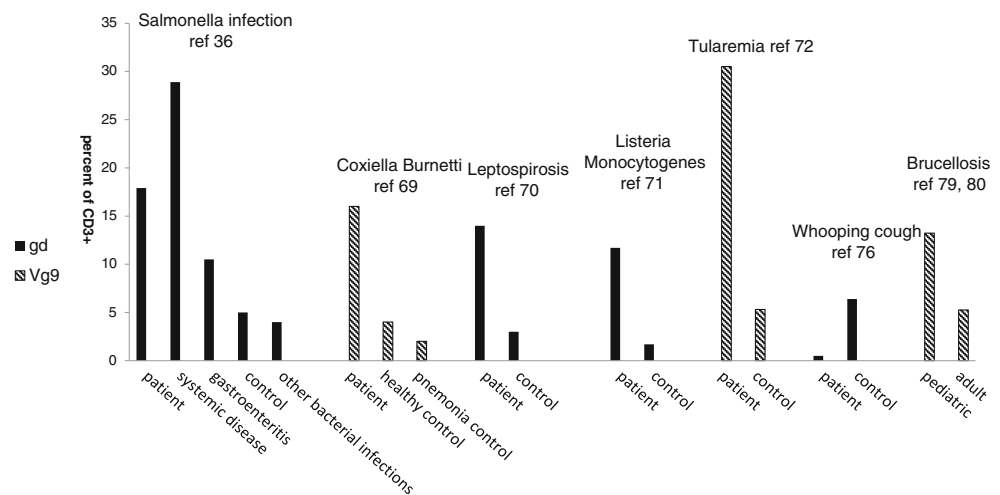
In four patients with acute *Coxiella burnetii* infection V $\gamma$ 9 $\delta$ 2  $\gamma\delta$  T cells increased to a mean of 16 % (range, 13–30 %) as compared with five healthy (mean, 4 %) and pneumonia controls (2 %; range, 1–3 %;  $P < 0.0014$ ) [69] (Fig. 5). In acute leptospirosis, a four- to fivefold increase in the number of PB  $\gamma\delta$  T cells was observed [70] (Fig. 5). Likewise, serial analyses of PBMC obtained from eight patients infected with *Listeria monocytogenes* showed a higher percentage of  $\gamma\delta$  T cells (median, 11.7; range, 3.7 to 35.3) than age-matched

**Fig. 4**  $\gamma\delta$  T cells in cytomegalovirus (CMV) infections after kidney transplants. Absolute number or percentage among peripheral blood CD3<sup>+</sup> T cells (secondary y-axis, bars indicated asterisk) of  $\gamma\delta$  T cell subsets based on references (ref) shown in the legend, along with subset measured, are shown. Plus sign and minus sign indicate patients with or without a CMV infection. H, healthy controls. m (number)= months after transplant of kidney



uninfected controls (median, 1.7; range 0.4 to 13) and patient's cells expressed significantly more HLA-DR [71] (Fig. 5). In 13 patients with tularemia, V $\gamma$ 9 $\delta$ 2+ cells represented an average of 30.5 % of the CD3+ cells, (nearly 100 % of the PB  $\gamma$  $\delta$ + T cells), on days 7 to 18 after the onset of disease whereas after vaccination with the live vaccine strain of *Francisella tularensis*, only a minor increase was noted [72] (Fig. 5). Likewise, in ulcero glandular tularaemia, % V $\gamma$ 9V $\delta$ 2 T cells within the first week of onset of disease did not differ from that of control subjects ( $5.3 \pm 0.8$ ) but did increase rapidly on days 8–40 (mean >20 %,  $P < 0.001$ ). Of 45 individuals sampled within 3 months of onset, 42 had >10 % of V $\gamma$ 9V $\delta$ 2 T cells. Moreover, significantly increased levels were still recorded at 18 but not at 24 months ( $10.2 \pm 2.1$  %;  $P > 0.10$ ) [73]. Similarly, among 14 subjects undergoing a Pontiac fever-like disease caused by *Legionella micdadei*, a pronounced increase of  $\gamma$  $\delta$  T cells occurred only 2 to 7 weeks after onset, with values slowly declining over 6 months, although without reaching the normal range [74]. In ehrlichiosis, in contrast, a course of antibiotic treatment (48–72 h) corrects the lymphocytopenia and is rapidly followed by a lymphocytosis of CD3+4-8- V $\gamma$ 9V $\delta$ 2+  $\gamma$  $\delta$  T cells [75]. Patients with whooping-cough by contrast had significantly lower relative and absolute numbers of PB  $\gamma$  $\delta$  T cells than normal controls [76] (Fig. 5). In two cases of psittacosis the number of  $\gamma$  $\delta$  T cell in the bronchoalveolar lavage fluid was markedly increased, declining to normal range along with recovery of their radiographical findings [77]. Finally, in pediatric bacterial meningitis,  $\gamma$  $\delta$  T cells producing IL-17 increased both in PB and cerebrospinal fluid, declining during therapy [78]. High levels of  $\gamma$  $\delta$  T cells, mainly V $\delta$ 2+, have also been seen in pediatric and adults patients suffering from acute brucellosis ( $13.23 \pm 4.7$  and  $5.25 \pm 1.4$ , respectively ( $P = 0.0001$ ) decreasing after treatment [79, 80] (Fig. 5). Taken together these data show strong and dynamic participation of  $\gamma$  $\delta$  T cells in infections, with measurable and pathogen-related patterns of responses.

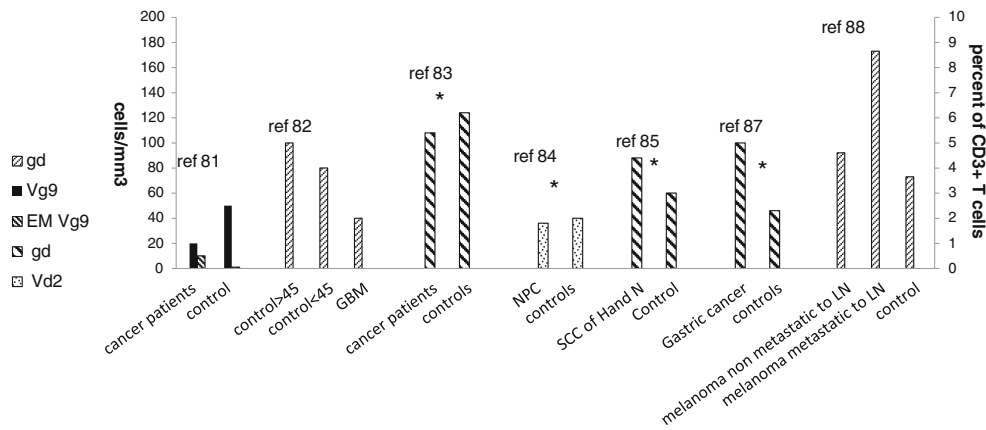
**Fig. 5**  $\gamma$  $\delta$  T cells in various bacterial infections. Mean percent of indicated  $\gamma$  $\delta$  T cells or V $\gamma$ 9+  $\gamma$  $\delta$  T cells subsets among peripheral blood (PB) CD3+ T cells, in indicated diseases, based on references (ref) above or to left of respective data bars



## Cancer

Because of the anti-tumoral effects of  $\gamma$  $\delta$  T cells, their potential therapeutic utility alterations of their subsets and correlation with cancer and its progression have been studied quite extensively. For example, the numbers of V $\gamma$ 9+  $\gamma$  $\delta$  T cells and in particular, CD27- CD45RA- effector memory V $\gamma$ 9+ subsets in PB were found to be significantly lower in 25 unselected advanced *solid tumor cancer patients* than in healthy subjects [81] (Fig. 6). Likewise  $\gamma$  $\delta$  T cell counts and mitogen-stimulated proliferative response of  $\gamma$  $\delta$  T cells were markedly decreased prior to *glioblastoma multiforme (GBM)* resection and throughout therapy (Fig. 6) [82]. However, others have not found a decrease of  $\gamma$  $\delta$  T cells or V $\gamma$ 9V $\delta$ 2 T cells in advanced cancer when analyzing small numbers of patients (Fig. 6) [83]. This discrepancy could be related to the nature of the alterations in the  $\gamma$  $\delta$  T cell subset in specific forms of cancer, and stage of the disease. For example late effector memory (CD27-CD45RA+) V $\gamma$ 9V $\delta$ 2 T cells (TEM RA) were significantly increased in *nasopharyngeal carcinoma (NPC)* with a corresponding decrease in central memory (CD27+CD45RO+) V $\gamma$ 9V $\delta$ 2 T cells (TCM), compared to healthy controls. Moreover, TEM RA and TCM V $\gamma$ 9V $\delta$ 2 cells from patients produced significantly less IFN $\gamma$  and TNF $\alpha$ , suggesting impaired cytotoxic potential (Fig. 6) [84]. On the other hand, in *squamous cell carcinoma* of the head and neck,  $\gamma$  $\delta$  T cells are significantly increased in PB (Fig. 6) [85]. Likewise, in *gastric cancer*, the frequency of V $\delta$ 1, but not V $\delta$ 2 or V $\gamma$ 9  $\gamma$  $\delta$  T cells, in the malignant tissue is significantly lower than in normal gastric mucosa and they produce significantly less IFN $\gamma$ , suggesting a defect of the V $\delta$ 1 subset specifically [86]. Conversely, the mean percentage of PB  $\gamma$  $\delta$  T cells in 48 gastric cancer patients and 49 controls was  $5.0 \pm 3.4$  and  $2.3 \pm 1.6$  % respectively; moreover, 41.7 % of the patients had a high percentage (greater than 5 %) of PB  $\gamma$  $\delta$  T cells compared to 8.2 % of the controls (Fig. 6) [87].





**Fig. 6**  $\gamma\delta$  T cells in cancer patients. Mean absolute number per cubic millimeter of peripheral blood (PB) or percent of indicated  $\gamma\delta$  T cell subsets among PB CD3<sup>+</sup> T cells (bars labeled *asterisk*, secondary y-axis), based on indicated references. *GBM*, glioblastoma multiforme;

*EM*, effector memory; *control* > 45 or *control* < 45 indicate age of controls; *NPC*, nasopharyngeal carcinoma; *SCC of H and N*, squamous cell carcinoma of head and neck; *LN*, lymph node

In American Joint Committee on Cancer (AJCC) stages I–III *melanoma*, the number of circulating  $\gamma\delta$  T cells was significantly increased compared with healthy individuals with  $\gamma\delta$  T cells producing TNF $\alpha$  or IFN $\gamma$  increasing after melanoma removal (Fig. 6) [88] [89]. In another study including 41 patients who underwent surgery for *renal cell carcinoma (RCC)*, 13 had stage III disease without metastasis. Those patients with peripheral  $\gamma\delta$  T cell greater than 8.7 % before surgery had better overall survival in a follow-up of 137 months. In contrast, five out of seven patients with a lower proportion of  $\gamma\delta$  T cells died [90].

#### Cancer Treatment

$\gamma\delta$  T cells bearing the V $\gamma$ 9V $\delta$ 2 TCR respond strongly to low molecular weight phosphorylated antigens in the mevalonate pathway, such as IPP, which are increased in cells by aminobisphosphonate inhibition of farnesyl pyrophosphorylase synthase (FPPS) [10]. Treatment of peripheral blood leukocytes with bisphosphonate reagents such as zoledronate (zol) or pamidronate thus leads to robust activation and proliferation of V $\gamma$ 9V $\delta$ 2 T cells. Synthetic reagents mimicking the physiological activators IPP have also been developed and they can activate and expand  $\gamma\delta$  T cells. Due to the anti-cancer cell potential of V $\gamma$ 9V $\delta$ 2 T cells, a number of clinical trials in which either V $\gamma$ 9V $\delta$ 2 T cell-stimulating agents or *ex-vivo* expanded V $\gamma$ 9V $\delta$ 2 T cells were used to treat solid tumor cancer have been designed.

Studies addressing  $\gamma\delta$  T cells in cancer patients who were treated with synthetic reagents mimicking the physiological activators (i.e., IPP) that activate and expand  $\gamma\delta$  T cells *in vitro*, e.g., reagent IPH1101 in combination with subcutaneous (SC) low-dose IL-2 revealed a potent  $\gamma\delta$  T lymphocyte expansion in 28 *solid tumor* patients [91]. However, in 12

patients with *metastatic RCC*, only a modest increase in V $\gamma$ 9V $\delta$ 2 T cell frequency could be detected by day 8 of therapy, in four of the nine patients who received at least one cycle of the bisphosphonate zoledronic acid together with low-dose IL-2 therapy. Furthermore, repeated administration actually diminished the *in vivo* percentage of V $\gamma$ 9V $\delta$ 2 T cells. In this study, although no objective clinical responses were observed by response evaluation criteria in solid tumor (RECIST) criteria, two patients experienced prolonged stable disease [92]. In ten therapeutically *terminal metastatic breast cancer* patients administered zol plus low-dose IL-2, treatment was well tolerated and promoted effector maturation of V $\gamma$ 9V $\delta$ 2 T cells. Seven patients who failed to sustain V $\gamma$ 9V $\delta$ 2 T cells showed progressive clinical deterioration, while three patients who sustained robust peripheral V $\gamma$ 9V $\delta$ 2 cell populations showed declining CA15-3 levels with one instance of partial remission and two of stable disease [93]. In another study, moreover, of 23 *breast cancer* patients, a single-dose (4 mg) of zol, with no follow-up of IL-2, induced a gradual and significant decrease of the different  $\gamma\delta$  T cell subsets, demonstrating a deleting effect of administration of zol in breast cancer patients [94]. In a phase I clinical trial in *metastatic hormone-refractory prostate cancer*, only two of nine patients treated with zol displayed a significant long-term shift of peripheral  $\gamma\delta$  cells toward an activated effector-memory-like state (TEM) associated with production of IFN $\gamma$  and perforin, compared to induction of this phenotype in most of the patients who were treated with zol and IL-2. Numbers of TEM  $\gamma\delta$  T cells significantly correlated with declining prostate-specific antigen levels and objective clinical outcomes (three partial remissions and five stable disease). However, most patients treated with zol alone failed to sustain  $\gamma\delta$  cell numbers and deteriorated clinically [95].

In another set of phase I/II studies, cancer patients received intravenous infusions of *ex vivo* expanded autologous  $\gamma\delta$  cells. In seven advanced renal cell carcinoma (RCC) patients receiving these infusions, prolongation of tumor doubling time was observed in three of five in whom an increase in the number of PB  $\gamma\delta$  T cells was achieved [96]. In another phase I study, of metastatic RCC, infusion of V $\gamma$ 9 $\delta$ 2  $\gamma\delta$  T lymphocytes combined with a low dose of SC IL-2 resulted in initial clearance of V $\gamma$ 9 $\delta$ 2 T from the blood, followed by their reappearance at the end of IL-2 administration [97]. An increase of  $\gamma\delta$  T cells was confirmed in *non-small cell lung cancer (NSCLC)* patients, whose PBMC were stimulated and expanded with zol (5  $\mu$ M) and IL-2 (1000 IU/mL) for 14 days *ex vivo*, followed by intravenous (IV) administration of harvested cells every 2 weeks. With increasing numbers of infusions, the number of PB  $\gamma\delta$  T cells gradually increased. Median survival was 589 days, median progression-free survival 126 days and no objective responses were observed. Six patients had stable disease, whereas 6 evaluable patients experienced progressive disease 4 weeks after the sixth transfer [98]. Finally, in a study of 25 patients with *various solid tumors* the numbers of CD3+ T cells, V $\gamma$ 9+  $\gamma\delta$  T cells and CD27– CD45RA– (memory) V $\gamma$ 9+ subsets in PB were significantly lower in patients than in healthy subjects. Encouragingly, numbers and frequency of CD3+ V $\gamma$ 9+  $\gamma\delta$  T cells subsets significantly increased in patients treated with infusions of *ex vivo* zol-activated and expanded V $\gamma$ 9+  $\gamma\delta$  T cells [81]. Together these studies show that pharmacological activation of CD3+ V $\gamma$ 9+  $\gamma\delta$  T cells is feasible in cancer patients, and may have clinical implications, but requires further investigation and refining.

Development of *cancer in post transplant patients* has also yielded important insights into the roles of  $\gamma\delta$  T cells in cancer. A longitudinal case–control study involved 18 solid organ recipients who developed cancer between 2 and 6 years after transplantation and 45 recipients who did not. The median percentage of  $\gamma\delta$  T cells among total lymphocytes in patients with malignancies was significantly lower compared with control patients at evaluations 6, 12, and 18 months before the diagnosis of cancer. In contrast, patients with a  $\gamma\delta$  T cell percentage of more than 4 % were protected. An increase of the V $\delta$ 2–  $\gamma\delta$  T cell subset significantly associated with lower incidence of cancer only in recipients who experienced pre- or postgraft CMV infection. Interestingly, moreover, a retrospective follow-up of 131 recipients for 8 years revealed that CMV-naïve recipients had an approximately fivefold higher risk of cancer compared with CMV-exposed patients [99].

**Hematologic Malignancies**  $\gamma\delta$  T cells (V $\gamma$ 9V $\delta$ 2+) were reduced in PB of *Burkitt Lymphoma* patients whereas in *follicular lymphoma*,  $\gamma\delta$  T lymphocytes were normally expressed in PB but less abundant in lymph nodes (LN) compared to inflammatory-reactive LNs [100] [101]. In newly diagnosed

*acute myeloid leukemia (AML)* absolute cell numbers of V $\gamma$ 9 $\delta$ 2 T cells were normal in both PB and bone marrow, but with a preponderance of the effector memory population [102]. In *acute leukemia* patients, increased cytotoxic V $\delta$ 1+ T cells were seen in 10 of 43 patients receiving anti- $\alpha\beta$  TCR monoclonal antibody (mAb) T10B9 T cell depleted (TCD) *bone marrow transplants* compared to only 7 of 100 of those receiving pan T cells mAb OKT3 TCD transplants ( $P=0.010$ ). Furthermore, the  $\gamma\delta$  T cells remained higher in the PB for a longer period of time. Patients with increased  $\gamma\delta$  T cells, whose grafts had been depleted with T10B9, showed a significant decrease in relapse, and estimated 3-year disease-free survival was significantly improved (0.79 vs 0.31,  $P=0.009$ ) [103].  $\gamma\delta$  T cell levels were also collected prospectively in 77 *acute lymphoblastic leukemia (ALL)* and 76 *acute myelogenous leukemia (AML)* patients undergoing partially mismatched-related donor allogeneic stem cell transplants (ASCT). Patients received a partially T cell-depleted graft using T10B9 ( $n=46$ ) or OKT3 ( $n=107$ ). Five-years leukemia free survival (LFS) and overall survival (OS) of patients with increased  $\gamma\delta$  compared to those with normal/decreased numbers were 54.4 vs 19.1 %;  $P<0.0003$ , and 70.8 vs 19.6 %  $P<0.0001$ , respectively, with no difference in graft versus host disease (GvHD) ( $P=0.96$ ). Thus,  $\gamma\delta$  T cells may facilitate a graft-versus-leukemia effect, without causing GvHD [104]

#### Immune-Mediated Diseases (Autoimmune, Auto-Inflammatory, and Allergic)

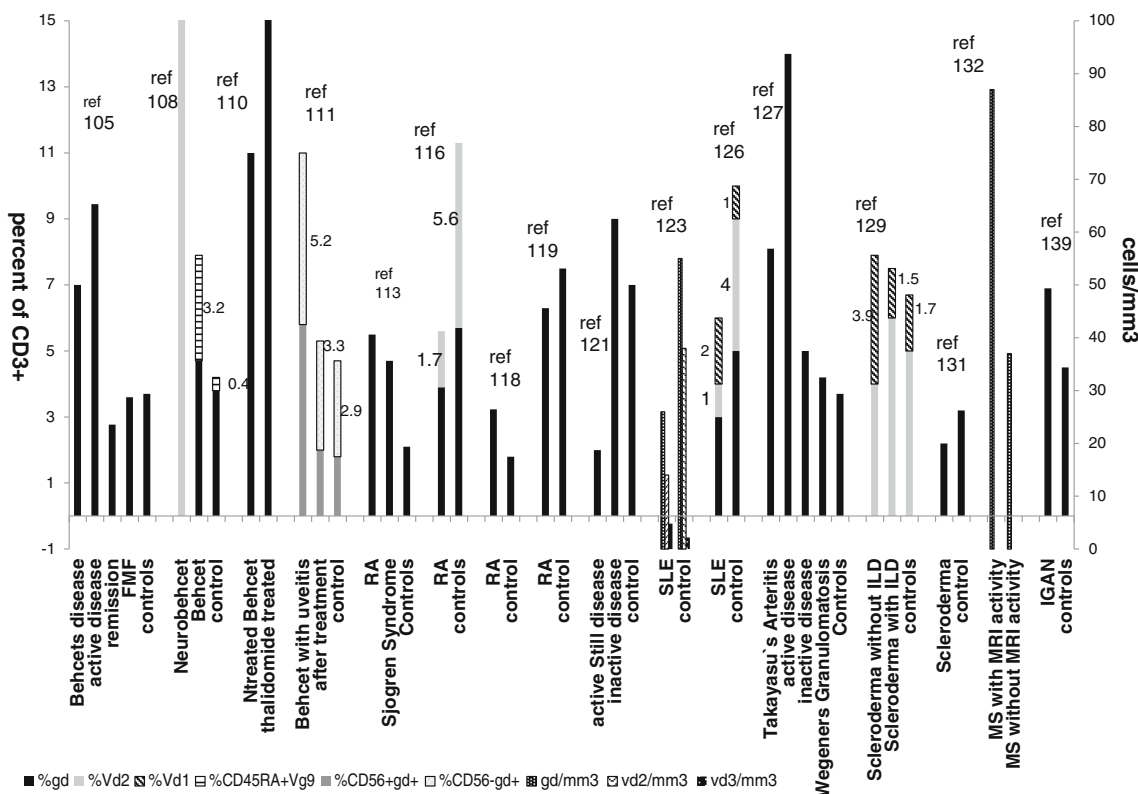
Due to their ability to secrete inflammatory cytokines, regulate dendritic cell and B cell functions and contribute to tissue destruction and repair,  $\gamma\delta$  T cells appear to play an important role in auto immune allergic and auto inflammatory diseases. Moreover, as reviewed below, different subsets may play unique roles in discrete diseases.

**Behcet's Disease (BD)**  $\gamma\delta$  T cells accounted for  $7.01\pm 4.42\%$  of the PBMCs in *BD* compared with  $3.56\pm 3.45\%$  in Familial Mediterranean Fever (FMF,  $P<0.005$ ) and  $3.7\pm 3.15\%$  in healthy individuals ( $P<0.009$ ) and their numbers were significantly higher during active disease than in remission ( $9.45\pm 5.08$  vs  $2.27\pm 3.3\%$ ;  $P<0.009$ ) (Fig. 7) [105]. Likewise, the increased  $\gamma\delta$  T cells (and CD8+  $\gamma\delta$  T cells) in *BD* patients exhibited increased expression of CD69, IFN $\gamma$  and TNF $\alpha$ , compared with healthy controls [106] [107]. Also, CD45RA+ V $\gamma$ 9V $\delta$ 2+  $\gamma\delta$  T cells, which constitute a minor population of  $\gamma\delta$  T cells in healthy individuals, were increased in number in *BD* irrespective of disease activity and, during active disease, co-expressed IL-2 receptors and HLA-DR (Fig. 7) [108]. Indeed, a significantly greater proportion of both V $\delta$ 1 and V $\delta$ 2  $\gamma\delta$  T cell subsets were more highly activated in active *BD* compared to inactive *BD* or healthy controls [109].

Surprisingly, in one open prospective study of ten patients given 200 mg/day thalidomide for 12 weeks  $\gamma\delta$  T cells further increased at day 90 (11 vs 21 %) after treatment (Fig. 7) [110]. In contrast, CD56+  $\gamma\delta$  T cells which were found to be significantly higher in active Behcet's uveitis, normalized after treatment (Fig. 7) [111]. Importantly,  $\gamma\delta$  T cells were only observed in the ulcerated oral mucosa but not in the non-ulcerated mucosa from BD patients or healthy controls with no preferential expression of specific V $\gamma$  or V $\delta$  chains [112]. Together, these reports indicate an important role of  $\gamma\delta$  T cells in the pathogenesis and immune regulation of BD.

**Auto Immune Arthritis; Rheumatoid Arthritis (RA), and Spondylarthritis** In one study,  $\gamma\delta$  T cells were significantly increased among the peripheral lymphocytes (5.5±4 %) in RA and patients with primary Sjogren's syndrome (SS) (4.75±3.85 %) as compared with healthy subjects (2.09±1.01 (P<0.001 for RA; P<0.004 for SS) (Fig. 7) [113]. A significant increase in the number of  $\gamma\delta$ -lymphocytes in the PB of patients with RA, especially in those with extra-articular manifestation of the disease, compared with normal controls, was likewise reported in a separate study [114]. On the other hand, significantly lower levels of  $\gamma\delta$  T cells were found by Smith

et al. in RA [115]. Another group likewise reported that  $\gamma\delta$  T cells, expressed as a percentage of CD3+ lymphocytes, were reduced in RA PB compared with a control group (3.9±0.5 vs 5.7±0.7 %; P less than 0.0001) most notably with a reduction in the V $\gamma$ 9 $\delta$ 2+ subset (from 5.6±1.2 to 1.7±0.4 %; Fig. 7) [116]. Data favoring a decrease in RA patients was further supported in a study of 17 psoriatic arthritis (PsA) and 16 RA patients who had, with respect to 27 healthy controls, lower values (both as percentages and in absolute numbers) of PB T cells expressing  $\gamma\delta$  TCR [117]. Liu et al. also found that RA patients had significantly lower levels of  $\gamma\delta$  T cells in PB than control subjects (1.38±1.08 vs 3.23±2.12 %, P<0.05; Fig. 7) [118]. However, in yet another study, the percentages (mean±SEM=6.3±0.8 %, n=22) and absolute numbers (70±11/μl, n=22) of  $\gamma\delta$  T cells in PB from RA patients were no different from 22 age-matched healthy controls (7.5±0.9 %, 81±17/μl, respectively). The  $\gamma\delta$  T cells in PB from 50 RA patients were, however, significantly decreased in negative correlation with the value of C-reactive protein (CRP) although they had no correlation with the titer of rheumatoid factor (Fig. 7) [119]. On the other hand, in PB lymphocytes of 12 patients with adult Still's disease, an unusual inflammatory systemic disease sometimes preceding RA, both the percentage and



**Fig. 7**  $\gamma\delta$  T cells in immune-mediated diseases. Mean percent of indicated  $\gamma\delta$  T cell subsets among CD3+ T cells (solid bars) or absolute number per cubic millimeter of PB (or refs. [123] and [132], secondary y-axis), based on references (ref) indicated just before respective data bars, in the diseases on x-axis are shown. IGAN, IgA nephropathy; SLE,

systemic lupus erythematosus, numbers indicate value for subset represented by adjacent bar inset; RA, rheumatoid arthritis; FMF, familial mediterranean fever. Inactive or active refers to listed disease. MS, multiple sclerosis; MRI magnetic imaging

absolute number of  $\gamma\delta$  T cells in active disease ( $n=6$ ) were significantly higher than during inactive disease ( $n=6$ ), inactive RA patients ( $n=8$ ), or healthy controls ( $n=20$ ) [120]. Curiously, in contrast, during *systemic juvenile idiopathic arthritis (JIA)* disease flare,  $\gamma\delta$  T cells were reduced (Fig. 7) [121]. These conflicting results suggest involvement of  $\gamma\delta$  T cells in RA, which may be related to disease activity and stage. Interestingly, the single study addressing  $\gamma\delta$  T cells in *ankylosing spondylitis (AS)*, revealed that the proportion of IL-23 receptor-expressing T cells in the periphery was twofold higher in *AS patients* than in healthy controls, specifically driven by a threefold increase of IL-23 receptor-positive  $\gamma\delta$  T cells [122].

**Systemic Lupus Erythematosus (SLE)** Absolute numbers of cells expressing  $\gamma\delta$  TCR in most of 32 *SLE* blood specimens were significantly lower than in the control group ( $n=16$ ) ( $P<0.006$ ) whereas mean values of the percentage of  $\gamma\delta$  T cells among pan T lymphocytes were almost the same (7.1 vs 6.3 %, respectively). Furthermore, no differences between the numbers of  $\gamma\delta$  T lymphocytes were observed between patients with active and inactive disease. However,  $V\delta 3+$  T cells were higher in *SLE* patients ( $20\times 10$  cells/ $\mu$ l) than in healthy controls ( $2\times 2$  cells/ $\mu$ l) ( $P=0.001$ ) (Fig. 7) [123]. Another study, however, reported a significantly decreased number of  $\gamma\delta$  T cells in *SLE* patients ( $26.4\pm 16.9/\mu$ l) compared with a control group ( $55.3\pm 20.6/\mu$ l) ( $P<0.001$ ) due to a decrease of  $V\delta 2$  TCR+ and  $V\gamma 9$  TCR+ subpopulations; however, no correlation between disease activity and the number of  $\gamma\delta$  T cells was demonstrated [124]. Reduced  $\gamma\delta$  T cells in *SLE* were found to express activation markers, CD69 and reduced CD94 [125]. Another group reported that a  $V\delta 1$   $\gamma\delta$  regulatory population, specifically decreased in the PB of *SLE* patients (Fig. 7) [126].

**Takayasu Arteritis (TA)**  $\gamma\delta$  T cells in 20 patients with *TA* were increased ( $8.1\pm 5.1$  %) compared to healthy controls, ( $n=20$ ,  $3.7\pm 2.1$  %,  $P=0.014$ ), RA ( $n=10$ ,  $4.8\pm 0.6$  %,  $P=0.032$ ), and Wegeners Granulomatosis patients ( $n=5$ ,  $4.2\pm 0.8$  %,  $P=0.030$ ). The increase was more prominent in active *TA* and associated with  $V\delta 1+$  cells, normalizing after 180 days of follow-up (Fig. 7) [127]. Immunopathologic analyses of vessel walls in *TA* revealed that the infiltrating cells consisted mainly of  $\gamma\delta$  T cells,  $\alpha\beta$  T cells and natural killer (NK) cells, which directly injured the vascular cells by releasing perforin [128].

**Scleroderma** One study showed that percentage of  $V\delta 1+$   $\gamma\delta$  T cells significantly increased among the PB T cells in *scleroderma* patients who did not have radiographic evidence of interstitial lung disease (ILD) ( $n=7$ ) compared to healthy controls and patients with ILD.  $V\gamma 9+$  T cells were equally and persistently represented irrespective of pulmonary disease or cyclophosphamide treatment, at levels similar to healthy

controls (Fig. 7) [129].  $\gamma\delta$  T cells in *scleroderma* were also reported to express CD69 to a greater extent than controls, indicating their activation *in vivo* [130]. Others, on the other hand have reported decreases of  $\gamma\delta$  T cells in *scleroderma* (Fig. 7) [131]

#### Auto-Immune Neurological Diseases

**Multiple Sclerosis** Among 20 *multiple sclerosis (MS)* patients presenting a first inflammatory event in the central nervous system, six showed intense magnetic resonance imaging (MRI) activity and had elevated CCR5+  $\gamma\delta$  T cells and total  $\gamma\delta$  T cells in the PB, which were not found in six patients without or with low MRI activity (Fig. 7) [132]. In addition, the percentage of CD16+  $\gamma\delta$  T cells was elevated in *MS patients* compared with healthy controls especially in patients with a progressive course of the disease and the extent of elevation positively correlated with time of disease progression and severity [133]. A recent study confirmed elevated  $\gamma\delta$  T cells among PB CD3+ T cells in relapse of *MS* and clinically isolated syndrome (CIS) patients ( $7.4\pm 5.2$  vs  $3.6\pm 2.8$  % in non-inflammatory disease controls), further showing specific elevation of the subset expressing CD161 and CCR6, markers associated with IL-17 production [134].

**Opsoclonus Myoclonus (OMS)** In two studies by Pranzatelli et al., the absolute but not relative size of the  $\gamma\delta$  T cell subset was reduced ( $-44$  %,  $P=0.02$ ) in paraneoplastic *OMS* [135]. In comparing cerebrospinal fluid (CSF) and blood of 36 children with *OMS* and 18 control subjects, most children with *OMS* had normal CSF cell counts but an expansion of  $\gamma\delta$  T cells (up to 26 %) subsets and a lower percentage of CD4+ T cells and CD4/CD8 ratio, which persisted even years after disease onset and conventional treatments. The percentage of activated CSF T cells was also higher. Abnormalities correlated with neurologic severity and disease duration [136].

**Guillain Barre Syndrome (GBS)** Serial flow cytometry studies revealed no significant difference in median  $\gamma\delta$  T cell percentages between *GBS* patients and controls at onset and at convalescence. However, five patients had marked  $V\delta 1+$  CD8+ elevations. Elevated  $V\delta 1$  or  $V\delta 1+$  CD8+ cells occurred in three of six patients with C jejuni or GM1 titer elevations [137].

**Miscellaneous Autoimmune Diseases** A significant decrease of  $\gamma\delta$  TCR+ cells in comparison to subjects after methimazole treatment as well as healthy controls has been observed in newly diagnosed *Graves' disease* patients. A significant increase of  $\gamma\delta$  TCR+CD8+ cells in the PB of subjects with *insulin-dependent diabetes*, treated with insulin for 3–6 months, has also been noted [138]. Finally, the proportion of  $\gamma\delta$  T cells in PBMC was higher in *IgAN* patients than in controls and

correlated with the proportion of surface IgA-positive B cells, which are precursors of IgA-secreting plasma cells (Fig. 7) [139].

### Atopic Diseases

**Asthma** The percentages of  $\gamma\delta$  T cells declined from 4.1 % in healthy to 3.2 % in allergic subjects and to a significantly lower 2.4 % in *allergic asthmatics*. The absolute numbers of circulating  $\gamma\delta$  T cells also were diminished in a similar fashion. In contrast,  $\alpha\beta$  T cells were comparable in healthy, allergic, and allergic asthmatic populations (Fig. 8) [140, 141]. Furthermore, in the PB of 26 adult patients with difficult-to-control asthma (DCA) and 22 patients with minimally symptomatic asthma (MSA) statistically significantly decreased relative and absolute numbers of  $\gamma\delta$  T cells ( $3.02 \pm 2.16$  % and  $0.06 \pm 0.04 \times 10(9)/l$ ), in comparison with controls ( $5.65 \pm 2.90$  % and  $0.13 \pm 0.08 \times 10(9)/l$ ), were found in the DCA patient group. Moreover, the relative and absolute numbers of  $\gamma\delta$  T cells were found to be diminished in both the allergy and nonallergy groups in comparison with healthy controls (Fig. 8) [142]. Interestingly, moreover, while smokers had elevated levels of PB  $\gamma\delta$  T cells, those developing COPD did not, suggesting that bronchial inflammation is associated with a relative decrease in  $\gamma\delta$  T cells in general [143]. Further support for a role of  $\gamma\delta$  T cells in asthma was demonstrated among 153 individuals, 95 with *controlled asthma* and 58 healthy controls, aged over 65 years. The  $\gamma\delta$  T cells were significantly decreased in the asthmatic patients compared to the controls (Fig. 8) [144]. Another study involving a group of patients with *atopic disease* including 11 children with *atopic dermatitis*, 20 with *atopic asthma*, and 18 adults with *atopic dermatitis* compared to 38 healthy age-matched controls aged 4–51 years, revealed, in the patients with atopic diseases, a significantly ( $P < 0.01$ ) lower proportion of  $\gamma\delta$  T cells in PB compared with healthy controls (median 4.8 vs 7.1 %), as well as significantly lower proportions of CD8+  $\gamma\delta$  T cells (Fig. 8) [145].

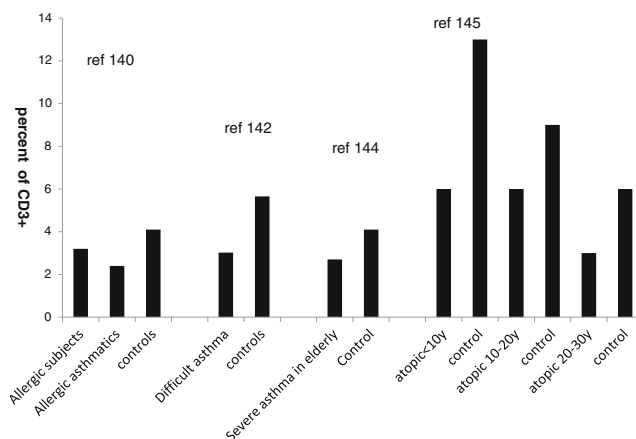
**Asthma–Bronchoalveolar Lavage (BAL) and Induced Sputum  $\gamma\delta$  T Cells** In induced sputum collected from ten patients with *acute exacerbation of asthma* and from healthy controls, a significantly decreased proportion of  $\alpha\beta$  T cells and an increased proportion of  $\gamma\delta$  T cells, CD56+ cells and CD8+  $\gamma\delta$  T cells were found in the asthma patients [146]. However, in *asthmatics*, no difference in the percentage of CD4+, CD8+, or  $\gamma\delta$  T cells in the BAL fluid before and after allergen challenge was observed. The major difference between the groups was manifested by an increased percentage of cells staining for the T helper-(Th)2-cytokines IL-5 and IL-13 in the  $\gamma\delta$  T cell subset [147]. In another study, BAL fluid of untreated *atopic patients* (six children and six adults) with mildly symptomatic chronic asthma was compared with BAL of ten healthy non-smoking volunteers and age-matched children with cystic

fibrosis ( $n=5$ ) or anatomic malformation of the airways ( $n=4$ ). The proportion of  $\gamma\delta$  T lymphocytes, primarily CD4+ or CD4- CD8- cells, was higher in asthmatic patients than in controls. Most lung  $\gamma\delta$  CD4+ lymphocytes expressed the  $\gamma\delta$  T cell receptor V $\delta$ 1 chain, proliferated in response to allergen stimulation, underwent steroid-induced apoptosis *in vitro*, and disappeared after systemic steroid treatment [148]. Thus, in asthma whether allergic or non-allergic,  $\gamma\delta$  T cells are decreased peripherally and increased in the lungs, suggest in their important role in this group of diseases in humans.

### Gastrointestinal and Liver Diseases

#### Liver Disease

$\gamma\delta$  T cells have been studied in Hepatitis B virus (HBV) liver disease, autoimmune hepatitis (AIH) and in cirrhosis. A decreased proportion  $\gamma\delta$  T cells was observed in acute or chronic, compared to normal and chronic HBV disease, and was inversely proportional to liver enzyme elevations (Fig. 9) [149]. In addition, the proportion of circulating V $\delta$ 2 T cells among all  $\gamma\delta$  T cells was significantly decreased in patients with chronic HBV infection compared to healthy individuals (Fig. 9) [150]. On the other hand,  $\gamma\delta$ T cells, in AIH patients were more numerous versus healthy controls, with an inverted V $\delta$ 1/V $\delta$ 2 ratio (Fig. 9) [151].  $\gamma\delta$  T cells also play a role in infectious complications in liver diseases.  $\gamma\delta$  T cells were increased in bacterially infected ascites fluid of cirrhotic patients relative to non-infected ascites [152], whereas in patients with end-stage liver disease (ESLD) prior to orthotopic liver transplantation (OLT), the median total lymphocyte count and both  $\gamma\delta$  T cells in general and the V $\delta$ 2+ subset in particular, at baseline, were significantly lower in patients with compared with those without infection [153]. Furthermore, after liver, as well as kidney,



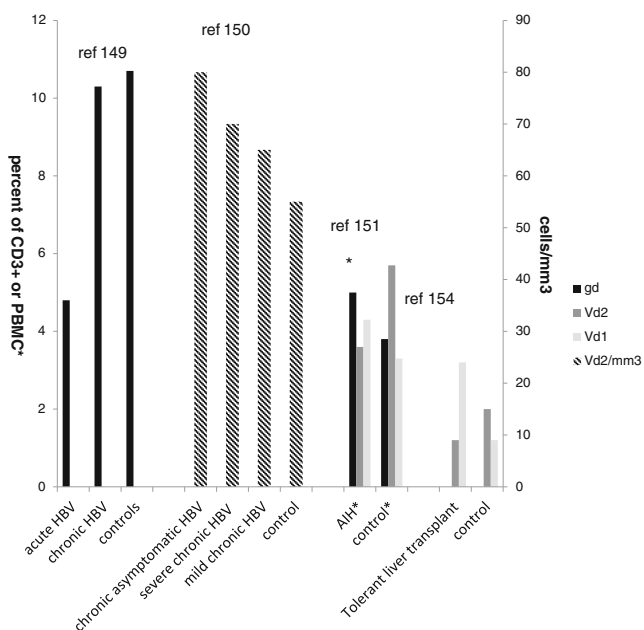
**Fig. 8**  $\gamma\delta$  T cells in allergic conditions. Mean percent, among peripheral blood (PB) CD3+ T cells, in allergic diseases indicated on the x-axis, of  $\gamma\delta$  T cell subsets based on references (ref) above or left of data bars. Y, years of age

transplantation, an increase of V $\delta$ 1 T cells occurred in PB of patients, in particular those with a stable transplant not requiring immunosuppression (Fig. 9) [154]

### Crohns Disease (CrD)

Conflicting results have been reported in CrD. In one study,  $\gamma\delta$  T subsets were lower in CrD patients (mean  $0.0259 \times 10^9/l$ ) versus healthy controls (mean  $0.0769 \times 10^9/l$ ,  $P < 0.001$ ), in particular the CD8+  $\gamma\delta$  T cell subset [155]. This decrease was associated moreover with an increase in the levels of IgE produced against *Encephalitozoon cuniculi*, a microsporidium whose presence in the gut was associated with CrD [156].

In contrast, others have reported that PB  $\gamma\delta$  T cells increased in patients with CrD compared with controls, with an elevated proportion expressing V $\delta$ 1 and V $\gamma$ 8—genes typically used by intraepithelial lymphocytes [157]. Lastly, a more extensive analysis of 46 patients found that most had  $\gamma\delta$ - T cells level comparable to healthy individuals (mean 2.2 %), whereas 24 % exhibited an increased level of  $\gamma\delta$  T cells (5–15 %) expressing V $\delta$ 2. In four male patients with a high baseline value, the  $\gamma\delta$ - T cell population increased dramatically following infliximab therapy and was oligoclonal [158]. Together, these studies suggest marked perturbations within the  $\gamma\delta$ - T cell repertoire in CrD that may be influenced by immunosuppressive therapy.



**Fig. 9**  $\gamma\delta$  T cells in liver diseases. Mean percent among peripheral blood (PB) CD3+ T cells or all PB mononuclear cells (asterisk) (solid bars) or number of cells per cubic millimeter of blood (hatched, secondary y-axis), in liver diseases indicated on the x-axis, of the indicated  $\gamma\delta$  T cell subsets based on references indicated above or to left of data bars. HBV, hepatitis B virus; AIH\*, autoimmune hepatitis

### Coeliac Disease (CoD)

Total circulating V $\delta$ 1+ lymphocytes among PB T cells were lower in 22 patients with untreated CoD compared with 16 healthy family members, whereas percentages of circulating CD45RO+ TCR  $\gamma\delta$  cells and CD45RO+ V $\delta$ 1+ cells were higher [159]. A series of studies addressed intraepithelial  $\gamma\delta$  T cells in CoD; patients had significantly higher levels of TCR $\gamma\delta$  intraepithelial lymphocytes than patients with other enteropathies, *Helicobacter pylori*-associated gastritis, and 37 normal control patients [160]. Likewise, in both children and adult patients, duodenal intraepithelial TCR  $\gamma\delta$  lymphocytosis significantly differs from healthy individuals (although more markedly in children. However, increased CD3+  $\gamma\delta$  T cells were diet and Marsh grade independent [161]. Indeed, increased  $\gamma\delta$  IELs exhibit a sensitivity of 93 % and specificity of 88 % for CoD [162]. Even reticulin autoantibody-positive children with normal jejunal mucosal morphology had significantly higher densities of intraepithelial  $\gamma\delta$ + T cells than antibody-negative ones [163].

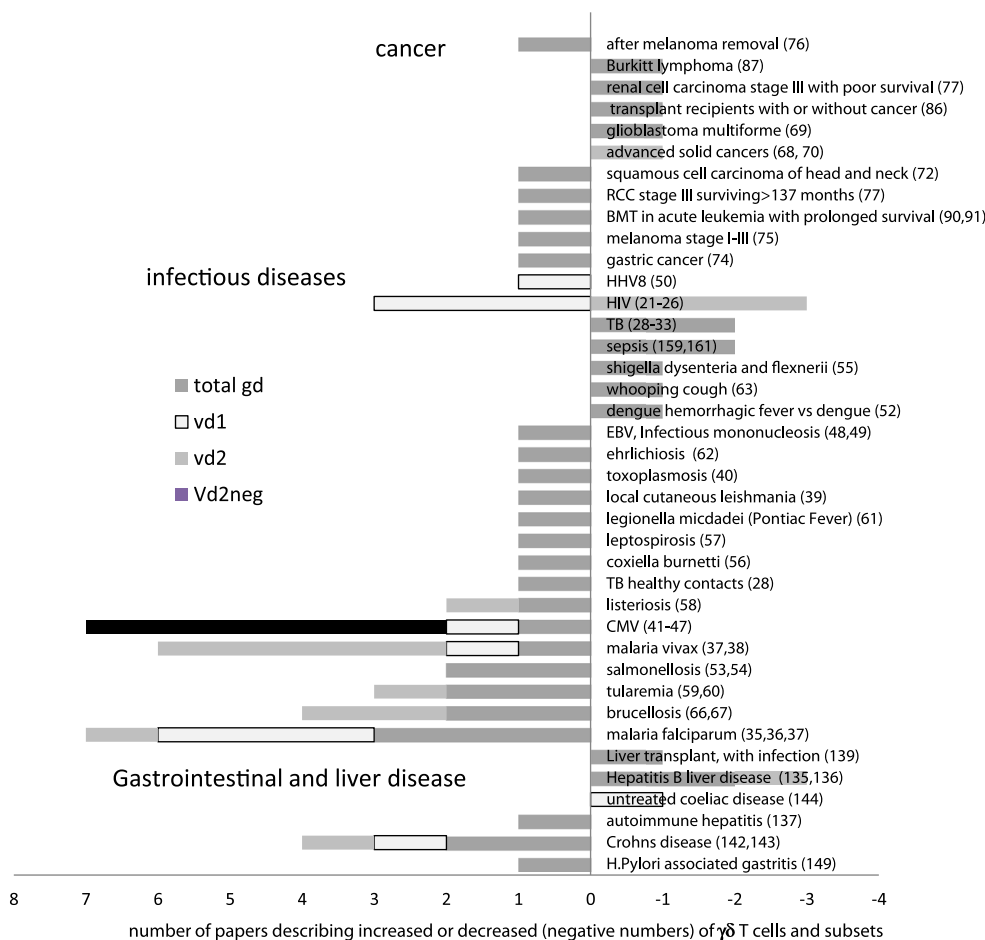
### Gastritis

$\gamma\delta$  T cell count in the mucosa was significantly higher in grade III gastritis with strong immunoglobulin (Ig)A and IgG responses to *H. pylori* urease than in grade II, I and normal mucosa.  $\gamma\delta$  T cell count significantly correlated with IL-1 $\beta$  and IL-7 levels in the gastric mucosa. An association was also seen between  $\gamma\delta$  T cell accumulation and *H. pylori* urease-specific Ig levels [164].

### Dermatological Disease

**Psoriasis** In psoriatic patients, a striking reduction of circulating V $\gamma$ 9V $\delta$ 2 T cells compared with healthy controls and atopic dermatitis patients has been noted, that normalized after successful psoriasis-targeted therapy. Furthermore, a distinct subset of pro-inflammatory cutaneous lymphocyte Ag, CCR6-positive V $\gamma$ 9V $\delta$ 2 T cells is rapidly recruited into the diseased skin, and V $\gamma$ 9V $\delta$ 2 T cells produced IL-17A and activated keratinocytes in a TNF $\alpha$ - and IFN $\gamma$ -dependent manner [165]. Other studies confirmed that the expression of TCR  $\gamma\delta$  was higher in guttate and plaque psoriasis than in normal skin [166] and that in psoriasis patients,  $\gamma\delta$  T cells were greatly increased in affected skin and produced large amounts of IL-17 [167].

**Atopic Dermatitis (AD)** In one study, the percentage of circulating V $\gamma$ 9V $\delta$ 2+ T lymphocytes was significantly increased in AD patients with respect to the age-matched controls, and a positive correlation with clinical score severity was found. Memory CD45RO+ CD62L+ V $\delta$ 2+ lymphocytes were significantly lower in AD patients. Furthermore, naive circulating V $\delta$ 2+ T lymphocytes were significantly lower



**Fig. 10** Summary of reported alterations in  $\gamma\delta$  T cells in disease. Increases (pointing left) or decreases (pointing right of upright axis) in  $\gamma\delta$  T cell and indicated subsets thereof, in physiological and disease conditions

grouped according to categories indicated on the right of each diagram are shown. The specific diseases or conditions are indicated on the right of the y-axis with appropriate references in parenthesis

in AD children than in age-matched controls. No correlation was observed between circulating V $\gamma$ 9V $\delta$ 2 T cell expansion and IgE serum levels [168]. In another study, however, the frequencies of circulating NK cells and  $\gamma\delta$  T cells were both profoundly reduced in AD patients [169].

**Miscellaneous Skin Diseases** In a *granulomatous syndrome*, due to a recessive genetic mutation in the TAP2 gene which is linked to the human leucocyte antigen (HLA) locus, both autoreactive NK cells and  $\gamma\delta$  T lymphocytes increased in the PB cells of two patients [170]. In *Bullous pemphigoid*,  $\gamma\delta$  T cells were reduced but rarely detected in lesional skin. In most patients, clinical remission and reduction of autoantibody titers after immunosuppressive therapy was not accompanied by an increase of circulating  $\gamma\delta$  T cells [171].

**Miscellaneous Medical Conditions**

**Stress** Granulocytes, macrophages, NK cells, extrathymic T cells,  $\gamma\delta$  T cells, and CD8+ subset were all found to increase

in the daytime (i.e., daytime rhythm) whereas, conversely total T cells, B cells,  $\alpha\beta$  T cells, and the CD4+ subset increased at night [172]. Both the V $\delta$ 1 and V $\delta$ 2 subsets were mobilized during stress, and for both subsets, TEMRA cells were mobilized to a much greater extent than the other memory phenotypes [173]. Acute psychological stress also significantly increased  $\gamma\delta$  T cells (5 to 5.55 % of CD3+ cells)[174].

During the severe systemic inflammatory response (SIRS) caused by trauma in 14 patients and by sepsis in 23 patients, the count of  $\gamma\delta$  T lymphocytes in the PB ( $30.1 \pm 6.0/\mu\text{l}$ ) was significantly lower than that of the healthy volunteers ( $104.3 \pm 10.9/\mu\text{l}$ ). The expression of CD69, an index of early activation of T lymphocytes, was significantly greater on  $\gamma\delta$  T lymphocytes from SIRS patients (patients  $23.9 \pm 3.4$  %, healthy controls  $4.8 \pm 0.6$  %,  $P < 0.05$ ). In the trauma patients, the expression of CD69 on  $\gamma\delta$  T lymphocytes increased rapidly within 48 h after injuries [175]. However, trauma patients undergoing *splenectomy* had a sustained increase in the percentage and/or absolute numbers of lymphocytes, CD8 T cells, activated CD8 T cells, NK T cells, NK cells, and  $\gamma\delta$

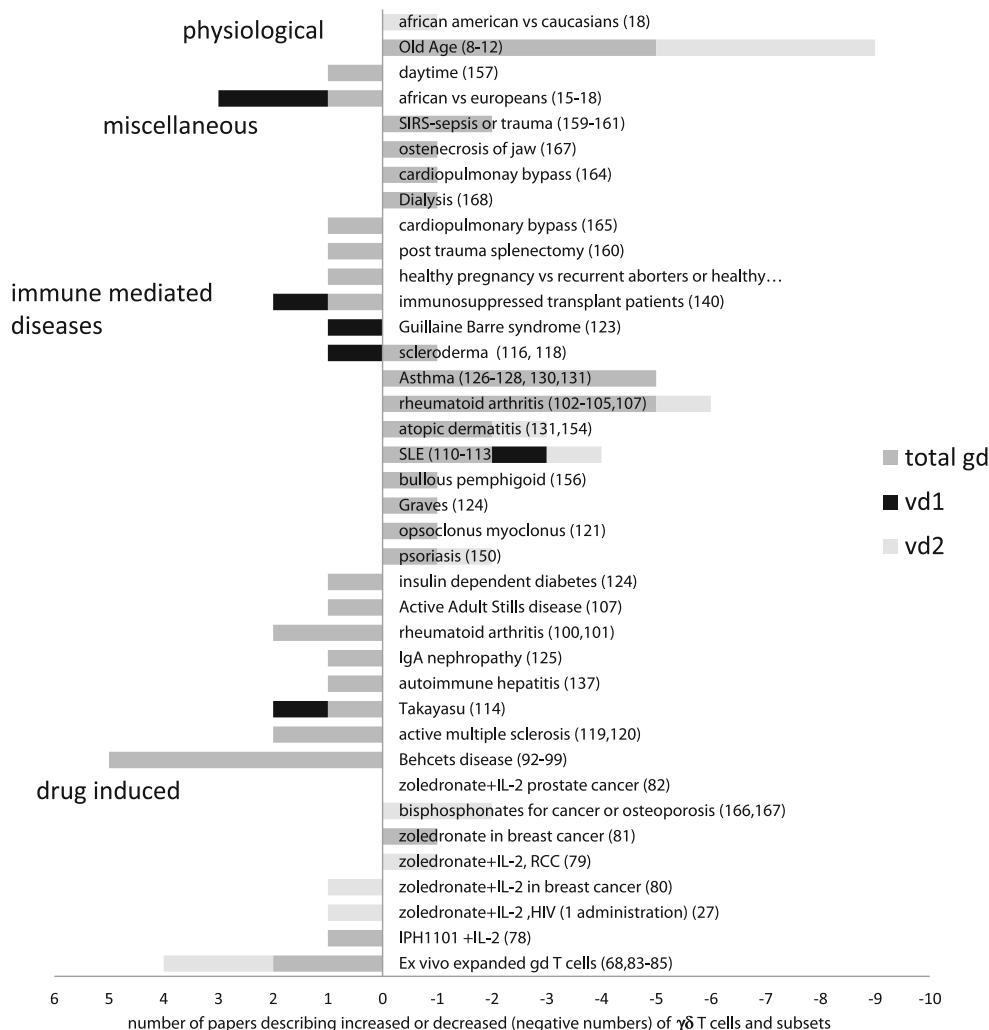


Fig. 10 (continued)

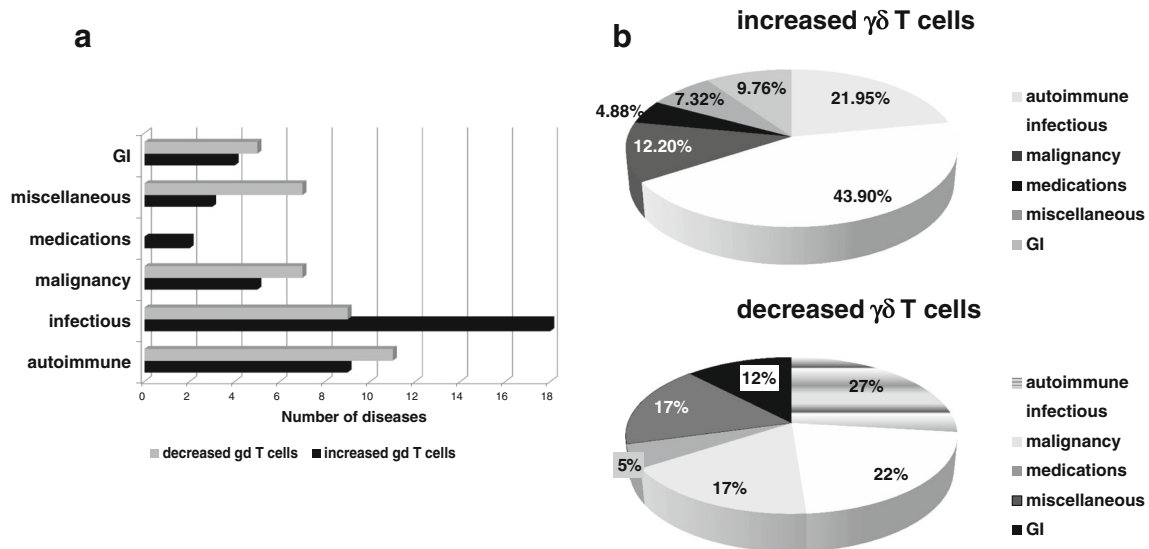
T cells, and a reduction in naive CD4 T cells [176]. In an adult intensive care unit in a university hospital, a study of patients with septic shock ( $n=21$ ) and healthy individuals ( $n=21$ ) likewise revealed a decreased percentage of  $\gamma\delta$  T lymphocytes in PB (1 % [0.7–3.1], median [interquartile range]) in comparison with the healthy individuals (3.5 % [2.1–4.8])[177]. Furthermore, lower numbers of  $\gamma\delta$  T cells, in particular of CD56+  $\gamma\delta$  T cells, in septic patients was the only marker (among all T cell subsets) significantly associated with death due to sepsis [178].

**Immunodeficiency** In recombinase-activating gene deficiency (RAGD) with residual V(D)J activity (>1 % recombination activity of wild type), several clinical and immunological subtypes have been described: RAGD with skin inflammation and  $\alpha\beta$  T cell expansion (classical Omenn syndrome), RAGD with skin inflammation and without T cell expansion (incomplete Omenn syndrome), RAGD with  $\gamma\delta$  T cell expansion and RAGD with granulomas [179].

**Transplantation** Phenotype, repertoire, and functional properties of  $\gamma\delta$  T cell subsets in a large population of allograft recipients were studied. Most immunosuppressed liver and kidney recipients displayed an enlarged PB  $\gamma\delta$  T cell pool mainly resulting from an expansion of V $\delta$ 1 T cells exhibiting an oligoclonal repertoire and different phenotypic and cytokine production traits than V $\delta$ 2 T cells [154]. In another study, the percentage of CD4 and CD8 T cells in transplanted patients was lower than in the control group ( $P<0.001$ ) with the exception of CD8  $\gamma\delta$  T cells from patients with stable evolution ( $P>0.05$ ) [180].

**Surgery** Blood samples from 24 children who underwent cardiac surgery with cardiopulmonary bypass (CPB) were collected serially to analyze TCR subsets by flow cytometry. The  $\alpha\beta$  T cells reached a nadir on postoperative day (POD) 1, but recovered to pre-CPB levels on POD 3. On the other hand, the  $\gamma\delta$  T cells decreased after CPB and did not recover to pre-CPB levels even after POD 7 [181]. In





**Fig. 11** Distribution of  $\gamma\delta$  T cell alterations among disease categories. **a** Number of diseases from Fig. 10 ( $n=82$ ), in categories designated on the upright axis, in which increased or decreased  $\gamma\delta$  T cells or their subsets in

peripheral blood (PB) have been described. **b** proportionate distribution of disease categories with increased ( $n=41$ ) or decreased ( $n=41$ )  $\gamma\delta$  T cells in PB

contrast, another study reported that the proportion of T cells bearing the  $\gamma\delta$  TCR as well as NK cells increased during CPB [182].

*Effect of Bisphosphonates* In volunteers receiving 5 mg ZA by intravenous infusion, a transient fall in circulating V $\gamma$ 9V $\delta$ 2 T cell levels at 48 h accompanied by increased serum levels of TNF $\alpha$ , IFN $\gamma$ , IL-6, and CRP were found in  $\geq 70$  % of the study volunteers [183]. Likewise, a notable loss of V $\gamma$ 9V $\delta$ 2 T cells occurred over time in osteoporotic patients on n-BP therapy, particularly those on IV therapy ( $n=68$ ) with no

difference in total T cells, monocytes or granulocytes. The observed negative effect on V $\gamma$ 9V $\delta$ 2 T cells coincided with the reported route of administration and timing of osteonecrosis of the jaw (ONJ). Six patients who had experienced ONJ were all significantly deficient in V $\gamma$ 9V $\delta$ 2 T cells (median=0.07 %) in comparison to age- and sex-matched treatment-naive controls ( $N=11$ ; median=2.40 %), and this was the only consistent difference in the leukocytes assessed. All ONJ cases had an underlying condition that further contributed to impaired immunity [184].

**Table 3** Examples of potential clinical use of  $\gamma\delta$  T cell enumeration in the peripheral blood

Prognostic	Measure	Ref	Diagnostic	Measure	Ref
Development of cancer in transplanted patients	$\gamma\delta$ , V $\delta$ 2-	[99]	differential of some intracellular infections from other causes of fever	V $\gamma$ 9V $\delta$ 2	[65–69, 79, 80, 159]
Prognosis in renal cell carcinoma	$\gamma\delta$	[90]	autoimmune vs viral hepatitis	$\gamma\delta$	[149–151]
Recurrence in leukemic patients after ABMT	$\gamma\delta$ , V $\delta$ 1	[103]	differential of malaria vs bacterial infections in travelers	$\gamma\delta$	[50, 51]
Development of brain lesions in multiple sclerosis	$\gamma\delta$ , CCR5+ $\gamma\delta$	[132]	in pregnant patients diagnosis of toxoplasmosis	V $\gamma$ 9V $\delta$ 2 CD45RA+ CD27-total $\gamma\delta$	[53]
Development of dengue hemorrhagic fever in dengue	$\gamma\delta$	[65]	differential of gastric from other cancers	$\gamma\delta$	[87]
Prognosis of infection in hepatic transplantation	$\gamma\delta$ , V $\delta$ 2	[153]	differential diagnosis of oligoarticular arthritis (e.g., Behcet vs Ankylosing Spondylitis)	$\gamma\delta$	[105, 122]
Development and resolution of cytomegalovirus infection in transplant recipients	V $\delta$ 2-	[57]	differentiating IgA nephropathy from Wegeners or lupus nephritis	$\gamma\delta$	[123–126, 139]
Prediction of osteonecrosis of the jaw during zoledronate treatment	V $\gamma$ 9V $\delta$ 2	[184]	differential diagnosis of bacterial sepsis from adult stills disease	$\gamma\delta$	[120, 178]
Death in sepsis	$\gamma\delta$ , V $\gamma$ 9V $\delta$ 2	[178]	Crohn's disease vs salmonellosis	$\gamma\delta$	[66, 67, 155]

**Hemodialysis** Peripheral  $\gamma\delta$  T cells were decreased in hemodialysis patients suggesting that circulating  $\gamma\delta$  T cells are susceptible to activation-induced cell death in comparison with  $\alpha\beta$  T cells in uremic environments [185].

**General Disease** Among 62 consecutive patients with >5 %  $\gamma\delta$  T cells of all PB T cells, more infectious, inflammatory, and autoimmune diseases were found than in controls [186].

## Summary and Discussion

This review of the published literature reveals 91 conditions and diseases (C&D) in which a significant alteration of PB  $\gamma\delta$  T cells and their subsets, compared to healthy individuals or other diseases, were reported as detailed in Fig. 10. Increases of  $\gamma\delta$  T cells were reported in 41 and decreases in 39 C&D (Fig. 11a). As shown in Fig. 11b, 43.90 % ( $n=18$ ), 21.95 % ( $n=9$ ), 12.20 % ( $n=5$ ), 9.76 % ( $n=4$ ), and 7.32 % ( $n=3$ ) of reported increase of the cells were accounted for by infectious, autoimmune, malignant, GI and miscellaneous diseases respectively, and 4.8 % by medications. On the other hand, 27 % ( $n=11$ ), 22 % ( $n=9$ ), 17 % ( $n=7$ ), 17 % ( $n=7$ ), and 12 % ( $n=5$ ) of the C&D with decreased  $\gamma\delta$  T cells were autoimmune, infectious, malignant, miscellaneous, or gastrointestinal, respectively, in nature and 5% of decreases were pharmacologically induced.

Disease-related increases or decreases of each of the  $\gamma\delta$  T cells subsets in PB presumably reflect a perturbation of the homeostatic mechanisms that normally govern the levels of >these cells in PB in health. Mechanisms involved could include activating effects of disease-related antigens of cytokines or of chemokines leading to cell proliferation or apoptosis as well as disease-related effects on migration to and from lymphatic or peripheral tissues. For example, the preponderance of infectious diseases associated with increased  $\gamma\delta$  T cells in the PB probably reflects, for the most part, the strong activating effects of phosphorylated antigens in the alternative mevalonate pathway of pathogenic bacteria on the V $\gamma$ 9 V $\delta$ 2 T cell subset [10]. In cancer and autoimmunity, antigenic influences are less well defined and are only recently being gradually uncovered. In these diseases, general dysregulation of the immune system via inflammatory and anti-inflammatory cytokines and chemokines produced in inflamed and malignant tissues presumably play an important role in the PB disease associated alterations of  $\gamma\delta$  T cells.

The possible pathogenic or protective significance of alteration of peripheral blood  $\gamma\delta$  T cells and their subsets should thus be viewed in the context of specific disease. For example, the strong activation of the V $\gamma$ 9 $\delta$ 2 T cells by pathogenic organisms might augment, on the one hand, clearance of the offending organism by  $\gamma\delta$  T cell mediated mechanisms, while inducing severe tissue damage mediated by cytokines released by

activated  $\gamma\delta$  T cells. In cancer, the reduction of anti-cancer cytotoxic  $\gamma\delta$  T cells observed in many instances might be supportive of cancer cell growth, whereas increased IL-17-producing  $\gamma\delta$  T cells might actually enhance tumor spread. Likewise, in autoimmunity  $\gamma\delta$  T cells could enhance inflammation, as well as induce apoptosis of proinflammatory and destructive cells.

Nevertheless, irrespective of the complex mechanism underlying the involvement of  $\gamma\delta$  T cells in each disease, analysis of the data collected here suggests that simple enumeration of these cells in PB of patients, albeit in the context of comparison to healthy age, sex, and race controls, may contribute, in the appropriate clinical setting, to diagnostic and prognostic evaluation of individual patients. For example, in the clinically common setting of a patient with undifferentiated peripheral arthritis, finding of elevated levels of  $\gamma\delta$  T cells would support a diagnosis of Behcet's disease, rather than of SLE. Likewise, elevated  $\gamma\delta$  T cells might favor a diagnosis of psittacosis over pneumococcal pneumonia in the appropriate clinical setting. Elevated  $\gamma\delta$  T cells in a patient with undiagnosed glomerulonephritis might support IgA nephropathy rather than lupus glomerulonephritis. Finding elevated V $\delta$ 2- T cells in a transplant patient may indicate the development of a CMV infection, whereas a decreased V $\gamma$ 9V $\delta$ 2 population may help differentiate psoriasis from atopic dermatitis. Elevated  $\gamma\delta$  T cells in bone marrow transplanted leukemic patients appear to suggest a better prognosis, and the same applies to development of cancer in solid transplant patients. On the other hand, finding a low level of  $\gamma\delta$  T cells in a cancer patient might enter clinical consideration of whether to administer zoledronate intravenously, since low levels of these cells are associated with increased chances of developing a devastating condition—osteonecrosis of the jaw. A summary of conditions in which differential diagnostic and prognostic value of enumerating peripheral blood  $\gamma\delta$  T cells may be potentially clinically relevant is presented in Table 3.

In summary, this paper represents an attempt to bring together, for the first time, data in the medical and scientific literature pertaining to the relationship of perturbations of  $\gamma\delta$  T cells in the peripheral blood of patients to medical conditions. Although most of the studies reported have as yet to be extended and substantiated, the current data suggests a strong potential for the clinical utility of a simple laboratory procedure—i.e., that of enumerating  $\gamma\delta$  T cells and their subsets in the peripheral blood—as a diagnostic and prognostic tool. Needless to say, much future study is required to substantiate this proposal.

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