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Tissue distribution, antigen specificity and effector functions of γδ T cells in human diseases

Gennaro De Libero

Experimental Immunology, University Hospital, Basel, Switzerland

Introduction

Since the discovery of T cells bearing the γδ TCR [1], a large number of studies have described the molecular characteristics of this receptor and examined the possible functions of these cells in a wide range of vertebrate species. However, although we know the genetics of γ and δ genes in great detail, the function of γδ T cells in vivo is only in part defined. In man, γδ T cells constitute ~5% of circulating $CD3^+$ cells and are most often $CD4^-8^-$ or $CD4^-8^+$ [2, 3]. Although γδ T cells represent a small population when compared to αβ T cells, they show peculiar functional characteristics that make them unique and important. Rather than discussing studies on the γδ T cells in general, this chapter will focus on the physiology of human γδ T lymphocytes and their possible role in diseases.

Gene organization of γ and δ loci

In the human genome the TCR γ locus spans 160 kb of DNA, and maps to chromosome 7. The γ locus comprises two constant gene segments and five joining elements, J1, JP, and JP1 located upstream of Cγ1 and JP2 J2, which are upstream of Cγ2. Fourteen variable γ (Vγ) genes have been identified, including eight pseudogenes and six active genes (reviewed in [4]). These genes fall into four subgroups designated VγI–VγIV. Five functional genes belong to the VγI family (Vγ2,3,4,5,8), whereas family II consists of only one gene, designated Vγ9 (Vγ2 in another nomenclature). Structural differences exist between Cγ1 and Cγ2 genes: Cγ1 is made of three exons, with exon 2 encoding the cysteine residue forming a disulfide bridge with the δ chain. Cγ2 has an allelic polymorphism with either two or three copies of an exon homologous to Cγ1 exon 2 without codons for this cysteine residue. Thus, γδ receptors using Cγ2 are not disulfide linked to the δ chain. On the cell surface at least four forms of γ

Correspondence to: Gennaro De Libero, Experimental Immunology, University Hospital, Hebelstrasse 20, 4031 Basel, Switzerland,
e-mail: Gennaro.DeLibero@unibas.ch

chain can be found: the 40-kDa γ1 chain, the differently glycosylated γ2 chains (of 40 or 44 kDa) encoded by the Cγ2 gene with the duplication of the exon 2, and the 55-kDa form encoded by the Cγ2 gene with the triplication of exon 2.

The human δ locus is within the TCR α locus on chromosome 14. One Cδ gene segment is located in front of the Jδ segment cluster and is preceded by four different Jδ segments. Three diversity (D) elements are also identified in front of the Jδ cluster [5–7]. The number of Vδ chains found expressed on the cell surface is limited to six [8]. A molecular map of the relative localization of Vδ genes indicates Vδ2 as the most 3' Vδ gene, followed in a 3'–5' direction by Vδ genes 8, 7, 5, 1, 6 and 4, while Vδ3 is located at 3' of Cδ [8].

The different TCR γδ which can be potentially expressed approximates to the large number of 10^{18}. The δ genes show an unprecedented junctional diversity with up to three Dδ segments used in tandem, together with imprecise joining and the extensive incorporation of N nucleotides [9]. Despite the small number of Vδ genes, these characteristics generate TCR δ chains with an extremely high variability in the CDR3 region, thus leading to a potential TCR γδ repertoire which is at least three orders of magnitude higher than the TCR αβ repertoire. This property may have important implications in the antigen recognition by γδ T cells.

Another striking feature of the TCR γδ is the preferential association of different Vδ and Vγ chains. Vδ2 mostly associates with the Vγ9 chain, while Vδ1 and Vδ3 chains mostly associate with Vγ chains other than Vγ9. This preferential association seems to be generated by some kind of antigen selection, since mixed combinations are found in γδ T cell clones isolated from thymus [10].

Maturation and selection in the thymus

Ordered and coordinated rearrangement of Vγ and Vδ loci in the human fetal thymus has been reported. The initial rearrangements join Vδ2 to Dδ3, and downstream Vγ genes (Vγ8 and Vγ9) to upstream Jγ gene segments. These rearrangements are characterized by minimal junctional diversity. At later developmental stages there is a switch such that Vδ1 is joined to upstream Dδ gene segments and Vγ genes are joined to downstream Jγ gene segments. These rearrangements are characterized by extensive junctional diversity [10, 11].

How γδ T cells differentiate in human thymus is not as clear as in the mouse. Mouse thymic γδ T cells are positively selected [12] and autoreactive γδ T cells are anergized [13] or deleted [14]. Positive selection is driven by cortical epithelial cells [15] and for some TCR γδ by MHC class I-like TL molecules [16]. The p56 lck protein tyrosine kinase, the CD45 protein tyrosine phosphatase [17] and the adapter SLP-76 [18] are necessary for intrathymic γδ T cell differentiation. MAP kinase activation is required for positive selection of αβ, but not γδ T cells [19]. IL-7 [20], IL-7 receptor [21] as well as IL-2 receptor beta chain [22] are required for maturation of γδ thymocytes. CD30 is required for negative selection of γδ T cells [23].

Maturation and selection in other organs

Mouse γδ T cells may also mature extrathymically. Intestinal maturation is inferred by the absence of Thy 1 antigen on mouse γδ T cells [24], and by the presence of im-

mature T cells in the gut of normal [25] and athymic mice (reviewed in [26]). Likewise in the human gut a minor population of cells has a phenotype of T cell progenitors. These precursor cells are $CD7^+$, $CD2^-$, $CD3^-$, $CD4^-$, $CD5^-$, and $CD8^-$ [27], and give rise to phenotypically mature αβ and γδ T cells.

TCR γδ transgenic or deficient mice have been instrumental in further understanding extrathymic maturation of these cells. CD3 ζ chain-deficient animals display highly reduced numbers of thymocytes, but normal numbers of intraepithelial lymphocytes (IELs), which however constitute a new population. These cells express normal levels of TCR-CD3 complexes associated with FcεRIγ homodimers. In contrast to CD3 ζ-containing IELs, these cells do not proliferate when triggered with anti-TCR mAbs or mitogens, suggesting that they mature in the intestine independently from TCR engagement [28]. In the intestine, precursor cells are $Thy\text{-}1^+$, $CD3^-$, $CD44^+$, $CD25^+$, $CD45R^-$, $CD24^+$. They mature into the γδ T cell lineage after expression of CD3ε chain and IL-2 receptor [29] and in the presence of locally produced IL-7 [30]. Expression of CD45 is also required for normal numbers of IEL γδ T cells. Indeed, in CD45 null mice apparently normal intestinal T cell maturation is followed by increased intestinal apoptosis and reduced numbers of IEL γδ T cells [31]. After maturation in the gut, γδ T cells become CD3 bright but are still functionally immature. They acquire a potent cytolytic activity only upon interaction with antigens and continuous stimulation [32]. Most likely antigen recognition induces an oligoclonal expansion of IELs, as suggested by the clonality of TCR sequences of intraepithelial, but not intra lamina propria T cells [33]. Extrathymic selection of γδ T cells has also been reported in the lung of mice [34]. These γδ T cells are positively selected by strain-specific polymorphic ligands that are encoded outside of the classical H-2 region. Selection can take place in the absence of thymus and requires IL-7 [35]. Maturation of γδ T cells may also occur in decidual tissue [36].

Distribution of γδ T cells in human tissues

A detailed analysis of the distribution of different Vγ and Vδ chains in various human tissues has been partially performed. Within the lymphoid organs, γδ T cells appear to be evenly distributed [2]. At variance with mouse, γδ T cells are rare in human skin, and form a minority of IELs in the gut. Nevertheless, human γδ T cells appear preferentially associated with epithelial cells, as seen in the mouse. In the tonsil they are mainly located in the interfollicular area, often around the small vessels and in the intraepithelial and sub epithelial zones. In these areas about 30% of cells express the TCR γδ [37], while very few γδ T cells are found in liver, kidney, salivary glands [38], and the bronchial tree [39]. In the thymus, which is the organ where most lymphocytes develop, γδ T cells are a very minor population, constituting less than 1% of total thymocytes.

The small number of available Vγ and Vδ segments are not used randomly and the TCR γδ repertoire is markedly restricted. The intestinal γδ T cells mostly use the Vδ1 and Vδ3 chains paired with members of Vγ1 gene family [40]. Nearly all the remaining γδ T cells in peripheral blood use Vδ1. Only a fraction of total thymocytes (less than 0.05%) express the Vγ9/Vδ2 heterodimer. On the contrary, Vγ9/Vδ2 T cells are very frequent in peripheral blood, in the tonsil and in the spleen, where they represent 5–10% of total lymphocytes and 50–80% of γδ T cells [10, 41]. Vγ9/Vδ2 cells are rare also in the placenta and in the peripheral blood of neonates [42]. Dur-

ing the first years of life these cells expand and become the predominant circulating γδ T cell population. This finding has suggested the possibility that environmental antigens stimulate and increase the number of these cells during the first years of life [42].

Antigen specificities of human γδ T cells

Despite intensive efforts, the antigens stimulating human γδ T cells remain in part unknown. Several types of antigen specificities have been reported. Here the most common ones, which are likely the most important, will be discussed first.

Recognition of non-peptidic phosphorylated antigens

Peripheral γδ T cells react with ligands present in many different microbes. Lysates from *Mycobacterium tuberculosis*, *M. bovis*, *M. leprae*, *Listeria monocytogenes*, *Staphylococcus aureus*, group A streptococci, *Escherichia coli*, *Salmonella typhi*, *Yersinia enterocolitica*, *Francisella tularensis* and *Plasmodium falciparum* induce proliferation of human γδ T cells (reviewed in [43]). In all these cases proliferating cells express the Vγ9/Vδ2 heterodimer.

The mycobacterial ligands have been extensively studied. They are protease-resistant non-peptidic compounds with a low molecular weight (<3 kDa) [44]. Constant et al. [45] isolated four different active compounds from *M. tuberculosis* cell extracts. One of these substances contains triphosphorylated thymidine linked to an unknown compound. Tanaka et al. [46] found that monoalkylphosphates may induce activation of Vγ9/Vδ2 cells and not of other γδ T cell clones bearing different Vγ and Vδ chains. In a third publication, it was reported that *M. tuberculosis* lysates contain active compounds with phosphate and an unidentified carbohydrate [47]. In later studies a ligand was purified from culture supernatants of *M. scrofulaceum* and *M. fortuitum* and characterized as a phosphorylated molecule without nucleotide residues [48]. This compound was identified as isopentenylpyrophosphate (IPP), which is an intermediate of prenyl and cholesterol biosynthesis and is present in both prokaryotic and eukaryotic cells [48]. At the same time we reported that IPP, together with five other naturally occurring non-peptidic metabolites, stimulate the same population of γδ T cells [49]. In addition, we isolated and characterized from *M. tuberculosis* cell extracts a non-peptidic molecule with a molecular mass of 262 daltons, containing a pyrophosphate but no nitrogen or cheton residues [43]. This molecule has an activity which is ~1000 times higher than IPP and could represent an intermediate in IPP synthesis in bacteria. By analysis of all active compounds reported so far it appears that the length and structure of the alkyl chain are important for immunogenicity. For example, while methylphosphate is active, dimethylphosphate and trimethylphosphate are not stimulatory [46]. We found that substitutions of IPP with a cheton in position 2 or with a hydroxyl in position 3 destroy immunogenicity. Moreover, the number and position of the phosphate groups also play an important role: while 2,3-diphosphoglyceric acid is active, neither 2-diphosphoglyceric acid nor 3-diphosphoglyceric acid are immunogenic (our unpublished data).

Recently, two new antigen reactivities have been reported for Vγ9/Vδ2 cells. Bukowski et al. [50] have shown that this population specifically recognizes several

Table 1. Ligands stimulating Vγ9/Vδ2 cells

Compound	Natural source	Structure identification	P groups	m.w.
TubAg1	*M. tuberculosis*	No	1	–
TubAg2	*M. tuberculosis*	No	1	–
TubAg3	*M. tuberculosis*	Partial	3	–
TubAg4	*M. tuberculosis*	Partial	3	–
MEP	No	Yes	1	126
Methyl-P	No	Yes	1	112
n-Propyl-P	No	Yes	1	140
iso-propyl-P	No	Yes	1	140
sec-Butyl-P	No	Yes	1	154
β-Hydroxyethyl-P	No	Yes	1	142
Phosphoglycolic acid	Yes	Yes	1	156
P-non peptidic	*M. smegmatis*	No	?	–
P-non peptidic	*M. fortuitum*	No	?	–
P-non peptidic	*M. tuberculosis*	No	?	–
IPP	*M. smegmatis*	Yes	2	216
IPP-CH_2OH	*M. fortuitum*	Partial	2	276
DMAPP	Yes	Yes	2	216
Farnesyl-PP	Yes	Yes	2	382
Geranyl-PP	Yes	Yes	2	314
Geranyl-geranyl-PP	Yes	Yes	2	450
Monoethyl-2'-dTTP	?	Yes	3	510
Monoethyl-2',3'-dTTP	?	Yes	3	494
Monoethyl-2'-dUTP	?	Yes	3	512
PP-non peptidic	*M. tuberculosis*	Partial	2	262
DPG	Yes	Yes	2	266
G-3-P	Yes	Yes	1	172
Xylose-1-P	Yes	Yes	1	200
Ribose-1-P	Yes	Yes	1	200
Ethylamine	Yes	Yes	0	45
n-Propylamine	Yes	Yes	0	59
n-Butylamine	Yes	Yes	0	73
iso-Propylamine	Yes	Yes	0	59
iso-Butylamine	Yes	Yes	0	73
sec-Butylamine	Yes	Yes	0	73
iso-Amylamine	Yes	Yes	0	87

P, Phosphate; MEP, monoethylphosphate; P-non peptidic, phosphorylated non peptide; IPP, isopentenylpyrophosphate; DMAPP, dymethylallylpyrophosphate; PP, pyrophosphate; 2'-dTTP, 2'-deoxythymidine triphosphate; 2',3'-dTTP, 2',3'-dideoxythymidine triphosphate; 2'-dUTP, 2'-deoxyuridine triphosphate; DPG, diphosphoglyceric acid; G-3-P, glycerol-3-phosphate

alkylamines released in millimolar amounts by different bacteria. Recognition of these compounds is mediated by the Vγ9/Vδ2 TCR and is affected by the type of the alkyl chain. Thus, alkylamines represent a new class of γδ ligands widely present in nature and capable of recruiting a large number of γδ T cells. A second reactivity is directed against bisphosphonates (pamidronate and alendronate), drugs usually used to contrast bone resorption. One of the most frequent side effects of this therapy is an acute-phase reaction, whose intensity appears correlated with expansion of the Vγ9/Vδ2 T cell population [51]. The mechanism by which these compounds induce proliferation of γδ T cells is not clear: they could directly mimic IPP and other pre-

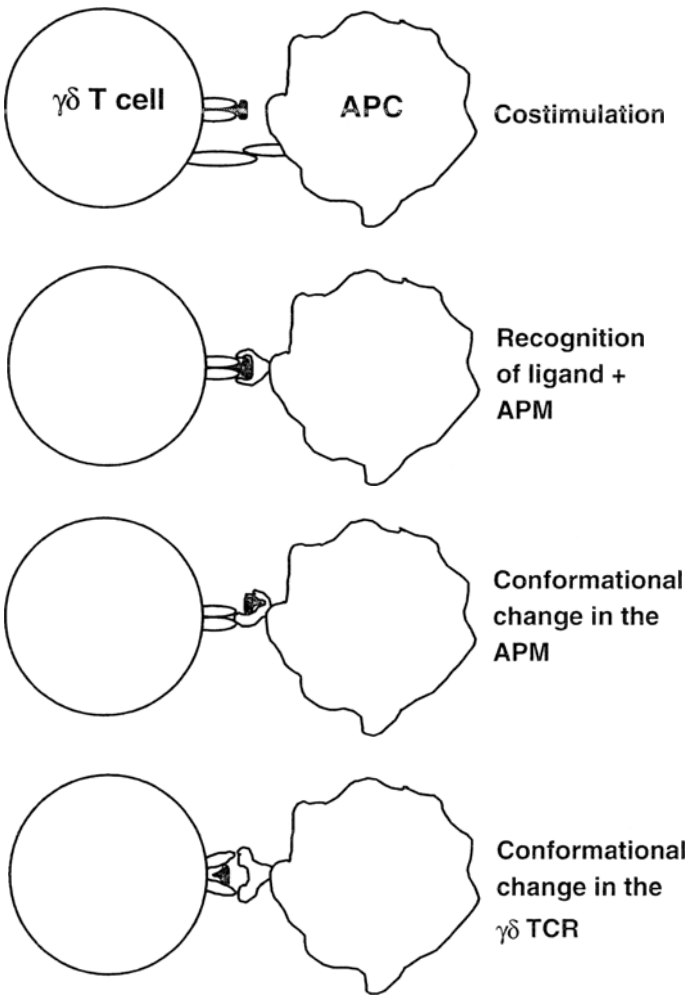

Fig. 1. Four possible models of interaction between γδ T cell receptor, stimulatory ligand and putative antigen-presenting molecule (*APM*) are shown (*APC* antigen-presenting cell)

nylphosphates, due to a structural homology, or instead induce accumulation of IPP and other metabolic intermediates since they block the mevalonate pathway [52]. Table 1 reports the active ligands described so far.

Analysis of several γδ clones pointed out that only cells expressing both Vγ9 and Vδ2 chains react to non-peptidic ligands [46]. In addition, the junctional regions of the Vγ9 or Vδ2 chains are important to confer reactivity to non-peptidic ligands [53], implying a reactivity different from that to protein superantigens and MHC-peptide complexes. However, as T cell clones with different junctional sequences cross-react with the same collection of ligands [49, 54], it has to be argued that many different γδ TCR recognize a structural motif common to all these compounds. An additional important observation is that a consistent fraction of Vγ9/Vδ2 thymocytes [55] or Vγ9/Vδ2 clones isolated from postnatal thymus [10] also react to this class of li-

gands, suggesting the possibility that this receptor has intrinsic structural characteristics which confer reactivity to non-peptidic ligands and their putative antigen-presenting molecules (APM). Whether dedicated molecules exist which present non-peptidic ligands to Vγ9/Vδ2 TCR is still an open issue. Filler cells and membrane-membrane interactions are definitely required to optimally stimulate γδ T cells [54, 56, 57] and only cells of human origin have this capacity (our unpublished results). These filler cells might provide necessary co-stimulation or, alternatively, they could associate non-peptidic ligands with species-specific APM, thus forming new complexes recognized by the Vγ9/Vδ2 TCR. Another possible way of interaction is initial binding of ligands to APC and induction of conformational changes of surface APM, which then make cognate interaction with the TCR. A speculative possibility is that non-peptidic ligands first bind to conserved regions of the Vγ9/Vδ2 TCR, which acquires a new conformation and the capacity to interact with APC. These four possible models of interaction are illustrated in Figure 1.

Recognition of cell surface molecules

Antigen reactivities of human γδ T cells against allo-MHC molecules [58–60], CD1c [61, 62], and CD48 [63] have been described. However, only a few γδ clones react to these molecules, suggesting that they are rare specificities. Also in the mouse a few alloreactive γδ clones were isolated and characterized. The reactivities of these cells are quite different from those of αβ T cells. One clone reacted to an unknown peptide presented by the TL 27b molecule [64], a second to the MHC class I-like TL 10b molecule [65], and a third to the MHC class II I-Ek surface glycoprotein [66], independently of associated peptides and antigen processing [67]. Site-directed mutagenesis of MHC molecules showed that the topology of γδ TCR interaction with the MHC is distinct from that of αβ T cells [67]. An intriguing finding is that mouse [68] and human [69] γδ T cells accumulate in the decidua during normal pregnancy. Mouse γδ T cells recognize non-MHC-encoded molecules present on both mouse and human trophoblast cell lines, but not other tumor cells [70]. The recognized surface structures have not been identified.

Recognition of peptides and carbohydrates

Rare human γδ T cell clones have been isolated which recognize peptide-MHC complexes [59, 71–73]. In mice there are also examples of these infrequent reactivities. Polyclonal γδ T cells specific for ovalbumin were induced after antigen inhalation [74]. It was not analyzed whether they were activated by the intact protein or by small peptides. A series of mouse hybridomas were reported to be stimulated by peptides derived from bacterial heat shock proteins (HSPs) [75]. Recognition of these peptides required presentation by unknown non-MHC molecules, and was affected either by amino acid substitutions in the peptide or by the polymorphism of Vγ chain.

Mouse γδ T cells may also recognize di- and trisaccharides coupled to class I-binding peptides [76]. The crystal structure analysis of these glycosylated peptide-MHC complexes has shown that the carbohydrates are located in the central region of the putative TCR binding site without altering the overall MHC structure [77].

These findings support a model of antigen recognition by these γδ TCR characterized by cognate interaction with the sugar residues and not with MHC molecules.

Recognition of heat shock proteins

A few studies have reported the possible recognition of HSPs by human γδ T cells [78–80]. In two of these studies a rabbit antiserum or an mAb specific for an unknown human 58-kDa HSP partially inhibited reactivity of freshly isolated, but not of cloned, γδ T cells to a tumor line stained by these antibodies. The HSP stimulating γδ cells was not identified and solid data supporting cognate interaction of the γδ TCR with members of this protein family have not been published. Taken together, all these findings show that antigen recognition by γδ T cells is different from that of αβ T cells. Additional evidence supporting this conclusion comes from the different length of CDR3 loops which are often critical for antigen binding in Ig and appear to provide the principal peptide binding residues in αβ TCRs. Comparison of the CDR3 regions of Ig H and L chains with TCR α, β, γ and δ chains, showed that Ig H and TCR δ CDR3 are the most variable in size and are significantly longer than Ig L and TCR γ chains, respectively. In contrast, TCR α and β chains pairing is constrained by nearly identical average CDR3 lengths [81]. These TCR structural differences have been related to γδ TCR recognition of molecules other than peptide-MHC complexes and, in general, more to a type of antigen recognition similar to that of Ig [82].

Effector functions of γδ T cells

Like αβ T cells, γδ T cells can be classified in different functional subsets. γδ T cell clones are potent cytotoxic cells which lyse different tumor cell lines [1] and secrete different lymphokines irrespective of the expressed V chains [3]. γδ T cells isolated from leprosy patients release soluble factors that positively influence development of granulomas [83]. In contrast, an inhibitory role in granuloma formation during mycobacterial infection has been attributed to γδ T cells in mice lacking the δ gene [84] or treated with anti-TCR γδ mAbs [85]. Another important function is protection during infections. By releasing proinflammatory lymphokines, γδ T cells might facilitate activation of macrophages in the early phases of infection. This has been described in mice infected with *Candida albicans*, in which γδ T cells facilitate nitric oxide production by macrophages and, thus, enhance resistance to mucosal candidiasis [86]. In addition, by killing the infected target cells, γδ T cells might contribute to microbial burden, as shown with cytotoxic $CD8^+$ T cells in mycobacterial and listerial infections in mice [87–89] or with human $CD8^+$ TCR $\alpha\beta^+$ T cells [90, 91].

γδ T cells may also regulate the effector phases of the immune response. After inhalation of soluble antigens, γδ T cells potently inhibit induction of IgE secretion in the mouse, possibly by blocking maturation of Th2 cells [74]. Thus, γδ T cells might have a prominent role in protection against primary allergic sensitization to environmental antigens.

γδ T cells may also influence the functional type of T helper cells which develop during immune responses. In mice infected with *Listeria monocytogenes*, γδ T cells produce Th1-type cytokines, while they produce Th2-type cytokines in *Nippostron-*

gylus brasiliensis-infected animals [92]. In both infections γδ T cells are among the first type of lymphocytes activated. As the effector phase of the immune response is influenced by the cytokine milieu in which the initial antigen priming occurs, γδ T cells may have the important regulatory role of determining the later response [92]. Furthermore, a possible role during viral infection has been proposed based on the findings that Vγ9/Vδ2 T cells recognize and kill target cells infected with different types of viruses [93].

An additional important function attributed to human γδ T cells is help to B cells. Indeed, it has been shown that, analogously to αβ T cells, human γδ T may facilitate B cell maturation, isotype switching and IgG production [94].

Role of γδ T cells in animal disease models

Studies conducted in mice deficient in γδ T cells by homologous recombination or treated with anti-TCR γδ mAbs have provided convincing evidence of the importance of γδ T cells in different disease models. γδ T cells provide protection during lethal encephalitis with HSV-1 [95], in malaria after immunization with inactivated sporozoites [96], and in the early phases of listeriosis [84, 97]. In the absence of γδ T cells, mice are more susceptible to high *M. tuberculosis* inocula [98] and have more severe tissue inflammatory injury after low inocula of the same bacteria [99]. In infection with *Nocardia asteroides*, γδ cells induce local recruitment of neutrophils responsible for microbial clearance [100]. In autoimmune disease models, γδ T cells appear to have an important regulatory role on αβ T cells and other effector cells. Elimination of γδ T cells worsens clinical manifestations in adjuvant arthritis [101] and lupus nephritis [102], leads to the aggravation and disease recurrence in experimental allergic encephalomyelitis [103], and mediates prevention of diabetes following mucosal insulin aerosolization in NOD mice [104].

Evidence for participation of γδ T cells in human diseases

Infections

Expansion of human γδ T cells has been described in bacterial, viral and parasite infections. The number of γδ T cells has been found increased in infections of bacterial origin such as brucellosis [105], salmonellosis [106], tuberculosis [107] and tularemia [108]. Increase of γδ T cells in hospital workers who are in close contact with tuberculosis patients has been also reported [109]. Increased numbers of circulating γδ T cells have been found in parasite infections such as malaria [106, 110, 111], ehrlichosis [112], leishmaniasis [113, 114], toxoplasmosis [115], and trypanosomiasis [116]. In most of these infections the expanded cells display a Vγ9/Vδ2 TCR.

The number of γδ T cells is also altered in some viral infections. In patients with AIDS a large number of oligoclonal $V\delta1^+$ cells are found in the peripheral blood [117] and of $V\delta2^+$ cells in the lung [118]. These γδ populations might contribute to the immune defense against opportunistic microorganisms frequently present in HIV-infected patients. Expansion of γδ T cells has also been found after cytomegalovirus (CMV) infection in kidney transplant patients [119]. The expanded cells express oligoclonal Vδ1 and Vδ3 chains and proliferate when challenged with CMV-infected

fibroblasts. These findings strongly suggest that an antigen-driven proliferation occurs during this viral infection.

The exact role of the expanded γδ populations in infections is not clear. γδ T cells might recognize microbial ligands and contribute to protection by reducing microbial load or by killing cells infected with intracellular pathogens. γδ T cells might also exert the important function of signaling the microbial presence in the early phases of infection, when a small load of microbial cells is present in tissues and other lymphocyte populations have not yet been recruited.

Tumors

One of the first identified functions of human γδ T cells was the "nonspecific" cytotoxicity toward tumor targets [1]. Moreover, some human γδ T cells kill Burkitt's lymphomas, thymic lymphomas and erythroleukemia cells [54, 120, 121]. Other studies identified γδ T cell clones reacting with unknown surface molecules expressed by activated B cells or EBV-transformed cells [122]. In all these cases no antigens interacting with the γδ TCR have been identified. Only a single γδ T cell clone that recognizes a peptide derived from the Ig expressed by a myeloma from the same patient has been described [123]. In some CNS neoplasms, including primary malignancies, metastatic cancers, and meningiomas, oligoclonal expansion of γδ populations was detected [124]. γδ T cells were found infiltrating cutaneous melanocytic tumors and capable of killing solid tumors [125]. γδ T cells may also accumulate around epithelial [126], renal [127] and lung [128] carcinomas.

A recent finding is that many tumors of epithelial origin express MICA [129]. Since this MHC-related molecule stimulates Vδ1$^+$ T cells [130], recognition of this surface antigen may allow γδ T cell response against tumor cells.

Autoimmune and inflammatory diseases

A possible involvement of γδ T cells in the pathogenesis of autoimmune diseases has been claimed. However, clear evidence of direct participation of γδ T cells in autoimmune reactions is not available. Most of the studies have analyzed the relative number of γδ T cells in peripheral blood or in other tissues, their phenotype and TCR repertoire. In rheumatoid arthritis the number of γδ T cells is increased in the affected joints [131], mainly in those with active synovitis [132]. In multiple sclerosis γδ T cells accumulate around brain lesions during the exacerbation phases [133] and in the cerebrospinal fluid where they display polyclonal Vδ1 chains [134, 135]. Expansion of γδ T cells has been reported in cases of autoimmune thrombocytopenia [136] and autoimmune neutropenia [137]. In this latter study it was reported that five affected patients showed monoclonal expansion of γδ T cells, thus suggesting a direct correlation with the development of neutropenia. Interestingly, clonal expansion of Vδ1$^+$ T cells was observed in an HTLV-I carrier patient with chronic neutropenia [138]. Taken together, these findings suggest that in some circumstances neutrophils might express surface molecules recognized by unique subsets of γδ TCR.

The number of circulating γδ T cells is increased in several inflammatory diseases. Coeliac disease (CD), an immune-mediated disorder arising from an hyper-responsiveness to gluten with histological alterations in the small intestine, is charac-

terized by increased numbers of intraepithelial and lamina propria γδ T cells showing a phenotype of activated and memory cells [139, 140]. However, increased numbers of γδ T cells are found also in patients with normal jejunal morphology or with latent disease [141]. Furthermore, the number of γδ T cells does not directly correlate with the presence of gluten in the diet [142]. According to all these observations, it is likely that γδ T cells do not react against gluten components. Interestingly, most γδ T cells in duodenum of CD patients express polyclonal TCR with predominant use of Vδ1, Vδ3 and Vδ8 chain [40], and the rare Jδ3 segment [143]. It is tempting to speculate that recognition of polymorphic molecules expressed by normal epithelial cells drives expansion of γδ T cells expressing these particular Vδ and Jδ gene segments. Candidate γδ T cell ligands are the MICA and MICB molecules, which activate Vδ1$^+$ T cells [130]. In CD, γδ T cells might have an anti-inflammatory role facilitating the repair of damaged tissue. The local protective role might be exerted by release of epithelial cell growth factors such as keratinocyte growth factor [144].

In inflammatory bowel disease (IBD), the number of γδ T cells has also been found to be increased in blood [145] and intestine [146]. Vδ1-Dδ3-Jδ1-bearing cells were found to be expanded in patients either with severe disease or in those with recently diagnosed or less severe forms of IBD.

Other inflammatory diseases showing increased numbers of γδ T cells are Still's disease [147], hypertrophic obstructive adenoids [148], Lyme arthritis [149], dermatitis herpetiformis [150], primary Sjogren's syndrome and untreated patients with systemic lupus erythematosus [151]. In chronic cutaneous lupus erythematosus Vγ9/Vδ2 T cells were observed in close vicinity to the damaged basal keratinocyte layer, suggesting their participation in the inflammatory reaction [152]. In lupus nephritis, γδ T cells help production of anti-DNA immunoglobulins [153]. In patients undergoing surgical interventions, there is infiltration of γδ T cells immediately after blood circulation is reestablished in ligated ischemic arteries, suggesting specific and immediate recruitment at the site of injury [154]. γδ T cells are also present in the transition zone between normal intima and fatty streaks in atherosclerotic plaques, suggesting a role in initiating the inflammatory process in atherosclerosis [155].

All these studies suggest that γδ T cells have multiple roles in autoimmune and inflammatory diseases. It is likely that in some cases γδ T cells participate in the pathogenesis of autoimmune diseases as they may recognize self antigens. However, it is conceivable that in other instances they may also limit inflammatory reactions and facilitate tissue repair.

Regulation of γδ T cells

The property of recognizing a large variety of non-peptidic ligands present both in eukaryotic and prokaryotic cells makes Vγ9/Vδ2 cells a readily activated cell population. Their overactivation may sometimes be responsible for dangerous acute inflammatory reactions, as reported in malaria infection [110] and following therapy with bisphosphonates [51]. Therefore, this type of antigen reactivity necessitates a tight control, which is likely provided by different mechanisms, leading to a fine balance between activation and inhibition of γδ T cells.

One regulatory mechanism is provided by weak agonist non-peptidic ligands which induce a state of transient anergy in γδ T cells after repeated stimulation. Natural compounds such as 2,3-diphosphoglyceric acid, which is present in huge

amounts (5 mM) in erythrocytes, induce unresponsiveness in γδ T cells to the most active ligands [156]. This state of anergy lasts for a few days and is associated with partial tyrosine phosphorylation of the CD3-TCR complex. All the Vγ9/Vδ2 T cells are affected by this inhibitory TCR engagement [156]. Thus, this mechanism may efficiently and simultaneously shut off the response of the whole Vγ9/Vδ2 T cell population, preventing its massive activation. Such a regulation may occur in patients constantly exposed to *P. falciparum*, e.g., individuals living in malaria-endemic areas who do not expand Vγ9/Vδ2 cells in vivo [157] and do not suffer from the pathognomonic acute inflammatory reaction concomitant to the blood stage of the disease.

A second regulatory mechanism is represented by expression of inhibitory receptors (IR) shared with NK cells. These receptors recognize MHC class I molecules and inhibit the response of γδ T cells more effectively when low rather than high antigen doses are present [158]. Engagement of IR facilitates recruitment of SHP-1 phosphatase to TCR-CD3 complex and affects phosphorylation of Lck and ZAP-70 kinase, but not of CD3 ζ chain upon TCR triggering [158]. These events may cause abortion of proximal TCR-mediated signaling. The role of IR is to set a higher TCR γδ activation threshold and therefore to focus the response of γδ T cells against APC loaded with high amounts of antigen.

In some cases, activation of γδ T cells requires facilitatory mechanisms. Optimization of γδ T cell response is important in tissues where a small number of γδ T cells is present and their effector functions have to be maximized to be effective. An enhancing mechanism is provided by expression of the CD66a molecule. This surface glycoprotein belongs to the CD66/CEA family and makes homotypic interactions with different CD66/CEA members. Engagement of CD66a with its physiological ligands enhances the amounts of released lymphokines, while it does not induce a shift of the dose-response curve (our unpublished data). Thus, it increases the potency of γδ response. As CD66 molecules are frequently expressed by epithelial cells, this interaction facilitates release of large amounts of lymphokines in epithelial tissues when small numbers of responding γδ T cells are present.

The function of γδ T cells in immune response

The enormous body of information derived from the reviewed studies allows us to discuss the role of γδ T cells in immune response on the basis of solid experimental data. A series of hypotheses can be made based on non-peptidic antigen specificity, on effector functions and also on cell number and distribution in human diseases.

The first function attributed to γδ T lymphocytes was that of sentinel cells according to their tissue distribution [159]. As γδ T cells in the mouse have the tendency to accumulate in the epithelia, it was suggested that this population may participate in the early host response against invading pathogens. A sentinel function has been attributed also to human Vγ9/Vδ2 lymphocytes according to their unique antigen recognition characteristics, rather than tissue distribution [43]. Vγ9/Vδ2 cells may fulfill the function of readily recruited and alerted sentinel cells, capable of immediately signaling the presence of danger. Their activation would have important consequences such as prompt release of pro-inflammatory cytokines and chemokines capable of facilitating the onset of local inflammation.

A second important function of γδ T cells may be to fill the gap between innate and acquired immunity, providing the response during the time when antigen-specif-

ic αβ T cells have not yet been recruited and expanded [43, 50]. The capacity of a series of ligands to activate a large number of γδ T cells, all sharing the same Vγ/Vδ pair, in a cross-reactive manner is unique for a lymphocyte population. This type of T cell activation is different from activation induced by peptides or bacterial and viral superantigens and, to some extent, it resembles ligand recognition by receptors expressed on the surface of innate immunity cells (e.g., CD14, CR1 and mannose receptor). In other words, Vγ9/Vδ2 receptors recognize patterns of molecules and do not make fine discriminations among them. In this respect, this subset of human γδ T cells exploits an antigen-recognition strategy typical of innate immunity cells, while at the same time retaining the functions of other lymphocytes.

A third important function is to drive maturation of antigen-specific T cells into different effector subsets. As shown during the early phases of infection in mice, the lymphokine secretion pattern of γδ T lymphocytes controls the subsequent functional maturation of αβ T cells [92]. Although there is no clear evidence that similar mechanisms also occur in human infections, it is likely that soluble factors released by human γδ T cells influence local lymphokine milieu. Therefore, it is conceivable that human γδ T cells also participate in the Th1 versus Th2 functional maturation of αβ T cells.

A fourth function of human γδ T cells is recognition of damaged or transformed epithelial cells expressing MICA and MICB proteins, whose gene transcription is regulated by promoter heat shock elements similar to those of HSP genes. As MICA and MICB are recognized by Vδ1-bearing cells, this subset may patrol the presence of recently altered cells in epithelial tissues.

Conclusions

In conclusion, the large number of studies on human γδ T cells have shown that these lymphocytes share several characteristics in common with αβ T cells, and also embody many unique properties. Some investigations have perhaps suffered a constant analogy with the αβ T cell population, which has precluded new and original experimental approaches. Nevertheless, this gigantic amount of work has provided solid clues for defining the role of human γδ T cells in diseases. In addition, we have also learnt more about the extreme plasticity of the immune system and its polymorphic capacity to adapt and recognize foreign molecules.

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