Lymphocytes: Gamma Delta

Julie Gertner, *INSERM, Toulouse, France*

Emmanuel Scotet, *INSERM, Nantes, France*

Mary Poupot, *INSERM, Toulouse, France*

Marc Bonneville, *INSERM, Nantes, France*

Jean-Jacques Fournié, *INSERM, Toulouse, France*

Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, Lymphocytes: Gamma Delta by Marc Bonneville and Jean-Jacques Fournié.

Advanced article

Article Contents

doi: 10.1002/9780470015902.a0001195.pub2

γδ Lymphocytes are a subset of T lymphocytes that express the γδ receptor for antigens. They are distinct from αβ T lymphocytes not only by their different mode of antigen recognition but also for the functions they fulfil. These HLA-unrestricted lymphocytes are particularly attractive for developing anticancer therapies based on new activatory drugs.

Introduction

Antigen recognition by B and T lymphocytes is achieved by highly diverse heterodimeric receptors, the B- and T-cell receptors (TCRs). T lymphocytes can be subdivided into two mutually exclusive subpopulations carrying TCR chains encoded by either α and β or γ and δ gene loci. These T-cell subsets are classically referred to as 'αβ' and 'γδ' T cells, respectively. Like other antigen receptors, the extensive structural diversity of γδ TCR is generated through somatic recombination of gene segments termed variable (V), diversity (D) and joining (J). Moreover, in common with αβ TCR, γδ TCR is noncovalently linked to a transduction complex composed of several CD3 subunits, which triggers intracellular signalling cascades and subsequent activation of T-cell effector functions following antigen recognition. **See also**: B Lymphocytes: Receptors; Immunoglobulin Gene Rearrangements; T-cell Receptors

γδ T cells, which were fortuitously discovered in humans and rodents about a decade ago, are produced by all vertebrates studied thus far. While they generally represent a small fraction of T cells in primary and secondary lymphoid organs, they are greatly enriched in mucosal tissues, where they can make up the vast majority of T cells. Despite this, whether γδ T cells fulfil functions distinct from, or redundant to, those achieved by αβ T cells has long remained an enigma. Although this issue is not yet solved, several observations detailed hereafter suggest that γδ T cells recognize a specific set of conserved antigens and play unique important roles in various immune responses and cell homeostatic processes. **See also**: Lymphoid System; Mucosal Lymphoid Tissues

Distribution, Morphology and Phenotype

Distribution

The relative abundance of T cells bearing γδ TCR varies greatly from species to species or from one body site to another. Furthermore, the individual's age and immunological history (e.g. infectious episodes) are two other important parameters that critically affect the frequency of γδ T cells in a given tissue location. In healthy individuals, γδ T cells represent an average between 1 and 5% of peripheral blood T cells in humans, and at most 3% of spleen and lymph node T cells in rodents. Their frequency in these body sites is much higher in ruminants and birds, where they constitute up to one-third of total T cells. The fraction of T cells expressing γδ TCR is greatly increased in epithelial sites directly contacting the external milieu. This enrichment is particularly pronounced in rodents, in which γδ T cells represent at least one-third of intraepithelial T cells throughout the digestive tract and in reproductive organ mucosa, and constitute most, if not all, intraepidermal T cells. An increased frequency of γδ T cells within intraepithelial lymphocytes of the digestive tract is also observed in humans and chickens, but their proportion in other epithelial sites remains a subject of debate. **See also**: Lymph Nodes; Spleen

Morphology

In peripheral blood, spleen or lymph nodes, mature γδ T lymphocytes are not distinguishable from either B or other (αβ) T lymphocytes by traditional

May–Grünwald/Giemsa staining. In their resting state, these cells frequently appear smooth and round, approximately 7–12 μm in diameter, with a central circular nucleus with a condensed amorphous chromatin appearance surrounded by a light-blue scanty cytoplasm. When activated *in vitro* or when drawn during viral or microbial infections, they appear, like activated αβ T lymphocytes, much larger with more irregular and granular shapes. These features are frequently shared by γδ T cells located in mucosa and stratified epithelia, where these cells can show a highly unusual 'dendritic-like' morphology, such as the murine intraepidermal γδ T cells, which for this reason have been referred to as 'dendritic epidermal T cells'. **See also**: B Lymphocytes; Dendritic Cells (T-lymphocyte Stimulating); T Lymphocytes: Helpers

Phenotype

Besides γ and δ TCR chains, which are by definition the most reliable markers allowing identification and isolation of γδ T cells, γδ T cells carry on their surface a large set of surface molecules whose expression can be detected by flow cytometry using a variety of labelled monoclonal antibodies. While most of these markers are shared with αβ T cells (e.g. CD2, CD3, CD7) and/or other haematopoietic cells (e.g. CD18, CD58), several are preferentially expressed by one or other T-cell subset. In rodents and humans, CD4 or CD8 coreceptors are found on the vast majority of mature αβ T cells but on a tiny fraction only of γδ T cells derived from nonepithelial sites. CD8 + γδ T cells are much more frequent in the blood and spleen of cattle and birds and in the intestinal mucosa of most animals studied thus far. However, unlike CD8 + αβ T cells, which generally express heterodimeric CD8 coreceptors (i.e. composed of an α and a β subunit), most γδ T cells express homodimeric 'αα' CD8 molecules, consistent with the thymic independence of some of these cells. There are several explanations for these phenotypic differences that are related to the distinct developmental features, antigen specificity and activation status of αβ and γδ T cells. In particular, the lack of both CD4 and CD8 coreceptors on most γδ T cells probably reflects their prominent reactivity against major histocompatibility complex (MHC)-unrelated ligands, as will be discussed later. Furthermore, the frequent expression of CD8αα homodimers by intestinal intraepithelial γδ T cells is probably due to their chronic *in vivo* stimulation. Indeed, expression of CD8 homodimers, unlike that of CD8 heterodimers, can be induced on various lymphoid cells, including γδ T cells and natural killer (NK) cells, following their *in vitro* activation. Moreover CD8 γδ T cells taken *ex vivo* display several phenotypic and functional features of preactivated/memory T cells such as CD25 or CD45RO. Unlike αβ T cells, γδ T cells frequently share several markers with NK cells, including the coactivatory NKGD2 homodimer, the FcγRIII receptor CD16 and CD56. Several inhibitory receptors binding to MHC class I (INMR), such as CD94/NKG2 heterodimers, are also frequently detected on blood NK and γδ T cells but seldom found on αβ T cells. The expression of INMR enables γδ T cells to modulate their activation threshold at signals received from the immunological synapse. Moreover, as for NK cells, INMR might help in controlling the inherent self-reactivity of some γδ T-cell subsets by delivering inhibitory signals upon interaction with self MHC class I molecules (see below). **See also**: CD Antigens; Flow Cytometers; Major Histocompatibility Complex: Human; Natural Killer (NK) Cells; T Lymphocytes: Cytotoxic

TCR Structure and Recognition Repertoire

Overall structure of the γδ TCR/CD3 complex

In common with αβ TCRs, γδ TCRs are heterodimeric receptors composed of two glycoprotein subunits, a rather acidic δ chain of 40–60 kDa and a rather basic γ chain of 30–60 kDa. Each TCR chain comprises two immunoglobulin-like domains. While the membrane-distal domain (termed variable or V domain) is structurally diverse, the membrane-proximal one (termed constant or C) is highly conserved from one γ or δ chain to another. The γδ TCR expressed by most circulating γδ T cells in humans presents an overall immunoglobulin (Ig) fold resembling to the αβ TCR, except its smaller elbow angle of the V:C domain border. This provides an orientation towards the side of the structure rather than to the top of the cell. The antigen-binding surface is not as flat as that of αβ TCR, and possibly accommodates ligands of small size. The short intracytoplasmic tail of TCR γ and δ chains is devoid of signalling properties, and thus transduction of the antigen-recognition signal requires additional transmembrane proteins, which are able to recruit intracellular second messengers. As for αβ TCR, this function is fulfilled by a multimeric complex comprising several CD3 subunits non-covalently linked to γδ TCR chains. The composition and stoichiometry of CD3 complexes expressed by most αβ and γδ T cells are very similar: these complexes possess six CD3 subunits (γ, δ, 2ε and 2ζ) forming homodimers (i.e. CD3ζζ) or heterodimers (i.e. CD3γε and δε). Interactions between the TCR chains and the CD3 complex are established through electrostatic bonds between polar residues located within the transmembrane regions of the TCR γ and δ and the CD3γ and δ subunits. Transduction of the signal is achieved by the CD3ε and ζ components, which carry one or several intracellular motifs (called ITAMs, immunoreceptor tyrosine-based activating motifs) that allow recruitment and phosphorylation of several tyrosine kinases such as p59fyn, ZAP70 and Syk, and subsequent activation of the whole signalling cascade. For reasons not yet understood, the γ chain of the type I receptor for Fcε (FcεRIγ) substitutes for CD3ζ in most intestinal epithelial γδ T cells and in a small fraction of splenic and lymph node γδ T cells. **See also**: Glycoproteins; Lymphocyte Activation

Signals: Transduction; Signal Transduction: Overview; Transmembrane Signalling

Genomic organization of γ and δ gene loci

The structural diversity of the TCR Vγ and Vδ domains is generated through somatic rearrangements of two (GV and GJ) and three (DV, DD and DJ) segments, respectively. Throughout the text, these segments are designated according to the last WHO-IUIS nomenclature (**Table 1**).

GV1S1–GV1S8 are referred to as Vg1–Vg8, respectively, according to Lefranc *et al*. or as Vg1.1–Vg1.8, respectively according to Yoshikai *et al*.

TCRG loci

The overall structure of the human TCRG locus, located on chromosome 7 at band 7p14–p15, is very similar to that of the TCRB locus. It comprises 14 GV gene segments belonging to four weakly homologous subfamilies. The first subfamily comprises eight members, of which only five are functional (GV1S2, GV1S3, GV1S4, GV1S5 and GV1S8). The second subfamily comprises a single functional V gene (GV2S1). All the other GV segments are nonfunctional. There are five GJ elements (GJ1S1, GJ1S2, GJ1S3, GJ2S1 and GJ2S2) located upstream of two GC genes (GC1 and GC2). Although the two GC genes are highly homologous between each other, they show several substantial structural differences. The GC1 gene comprises three exons: exon I codes for the extracellular Cγ domain; exon II for the 'connecting' region which comprises a cysteine involved in a TCRγ/δ interchain disulfide bridge and exon III which codes for the hydrophobic transmembrane region and the short intracytoplasmic tail. The GC2 gene contains four or five exons owing to duplication or triplication of exon II. Moreover, since exon II of the GC2 gene is devoid of cysteine, there is no covalent link between TCR δ chains and TCR γ chains encoded by TCRGVJC2 genes.

The murine TCRG locus, located on chromosome 13, is organized in 'clusters'. There are four GC genes (GC1, GC2, GC3 and GC4), each preceded by a single GJ element and one or several GV elements. These clusters are rearranged independently and sometimes concomitantly on the same chromosome, thus in some cases, leading to expression of distinct TCR γ isotypes paired with the same TCR δ chain in a given T-cell clone. This lack of 'isotypic exclusion', which is reminiscent of the lack of allelic exclusion of TCR gene rearrangement and expression observed in some αβ and γδ T-cell clones, does not seem to have any dramatic physiopathological consequences.

TCRD loci

In both humans and mice, the TCRD locus is embedded within the TCRA locus on chromosome 14, between the AJ and AV elements. It comprises two (in mice) or four (in humans) DJ elements, followed by three DD elements and a single DC gene. One DV segment (DV103S1 in humans, DV105S1 in mice) is located downstream of DC in an inverse transcriptional orientation. All the other DV segments are interspersed within the AV segments, upstream of the DD elements. Because of the peculiar organization of the TCRA/D loci, any AV or DV segment could in theory be used to form a TCR α or δ chain. Accordingly, some V segments, such as DV101S1 in humans, are found equally on δ and α TCR chains. However, this seems to be an exception rather than a rule. Actually only 15 (out of approximately 50 functional) V segments have been found rearranged to DD/DJ elements, and only 3–5 of these V elements (DV101S1, 102S1, 103S1 in humans, DV101S1, 102S1, 104S1, 105S1 and ADV7S1 in rodents) have been detected on a significant fraction (above 1%) of γδ T cells. The precise rules governing usage of a given V gene by either αβ or γδ T cells are still unknown: in addition to central and peripheral antigen selection processes (see below), they probably involve several structural features of the V domains (e.g. VαVβ/VγVδ pairing constraints) or of the V genes, such as V gene location and accessibility to recombinases, compatibility of the V/J recombination signal sequences and so on.

Junctional diversity of γδ TCR

Despite the relatively small number of distinct V(D)J elements available for recombination, the sequence diversity of γδ TCR chains is considerable, due to extensive nucleotide trimmings and additions occurring at the junctions of the rearranging V(D)J segments. This is particularly true for TCR δ chains, which are encoded by up to five elements rearranged in tandem (i.e. VD1D2D3J) and thus contain up to four sites of nucleotide insertion/nibbling. Furthermore, D segments can be read in all three reading frames. Hence, based on theoretical considerations, the size of the γδ TCR repertoire has been estimated as up to 10^{17}, as compared to 10^{15} for αβ TCR. However, for several reasons detailed below, the actual size of the γδ T-cell repertoire and the diversity of their recognized antigens is much more limited.

Tissue-dependent restriction of the γδ TCR repertoire

A striking and unique hallmark of γδ T cells is the preferential expression of different V regions in distinct tissue locations, thus suggesting recognition of a restricted set of related antigens in a given body site which would differ from one tissue to another (**Table 1**). In humans, up to 95% of peripheral blood γδ T cells use a V gene combination (GV2S1/DV102S1), which is seldom found in other tissues (e.g. in spleen, thymus and intestinal epithelium). Interestingly, the proportion and absolute number of peripheral blood γδ cells bearing GV2S1/DV102S1 + TCRs are low at birth but then rapidly increase in this site during the first years of life, while they remain low and stable in other sites (e.g. in thymus). In parallel, blood GV2S1/DV102S1 + γδ T cells acquire several markers of

Table 1 Gamma delta T-cell subsets in the mouse and human

Species	TCR V regions	Main previous TCR designation	TCR diversity	Developmental origin	Preferential location
Mouse	GV1S1/DV101S1 (GV5S1/DV101S1)	Vγ3/Vδ1[a] Vγ5/Vδ1[b]	0	Fetal	Epidermis
	GV2S1/DV101S1	Vγ4/Vδ1[a] Vγ6/Vδ1[b]	0	Fetal	Vagina, uterus and oral epithelium
	GV5S1/DV104S1 or ADV7	Vγ1.1/Vδ4 or 6[a] Vγ1/Vδ4 or 6[b]	+ / + +	Fetal/adult	Intestinal epithelium, spleen, lymph node
	GV4S1/diverse Vδ	Vγ5[a] Vγ7[b]	+ +	Adult	Intestinal epithelium
	GV3S1/diverse Vδ	Vγ2[a] Vγ4[b]	+ + +	Adult	Spleen, lymph node
Human	GV2S1/DV102S1	Vγ9/Vδ2[c] Vγ2/Vδ2[d]	+ / + +	Fetal/adult	Peripheral blood
	Diverse Vγ/DV101S1 or DV103S1	Vδ1 or Vδ3	+ + / + + +	Fetal/adult	Spleen, intestinal epithelium

TCR V regions have been designated according to the official World Health Organization nomenclature, which is seldom used in the literature.
[a]Raulet (1989).
[b]Haas and Tonegawa1 (1993).
[c]Lefranc (1990).
[d]Yoshikai *et al.* (1987).

memory cells, indicating an ongoing antigen-driven expansion. Hence, these findings suggest that the skewed V gene usage by peripheral blood γδ T cells is the consequence of a postnatal peripheral selection process mediated by a restricted set of highly recurrent antigens. The biased usage of particular V regions is also observed in other peripheral sites such as spleen and intestine, where human γδ T cells express predominantly DV101S1 + or DV103S1 + chains. Since the diversity of the TCR chain junctional sequences and of the Vγ regions expressed by these cells is quite important, it suggests their *in vivo* selection by a rather heterogeneous set of antigens.

Accordingly, recent studies have demonstrated that a mouse γδ TCR contacts directly its T22 MHC class I-like ligand by the D-encoded amino acid residues. In this species, three sets of γδ T cells are usually distinguished. One set matures in fetal thymus and then homes to defined epithelia with a correlated expression of homogeneous ('invariant') TCR comprising GV1S1/DV101S1 or GV5S1/DV101S1 regions in the skin and GV2S1/DV101S1 regions in the mucosa of vagina, uterus and tongue (vut-IEL). These populations represent extreme examples of tissue-dependent repertoire restriction because not only do they express similar V region combinations in a given site but also their TCR chains show identical junctional sequences. This extensive TCR sequence conservation is partly due to enzymatic constraints, as all these subsets are derived from fetal precursors that are devoid of terminal-desoxynucleotidyl transferase, and thus cannot diversify their V(D)J junctions by 'N' nucleotide additions. However, there is strong evidence for an additional selection of these cells by highly conserved but yet undefined epithelial ligands that further restricts the sequence diversity of the TCR clonotypes expanded *in situ*. A second set of murine γδ T cells matures in adult thymus, locates in all lymphoid organs and expresses diversified TCR comprising mainly GV5S1 or GV3S1, and sometimes GV5S2 or GV4S1-encoded V regions. A third group matures extrathymically, expresses diversified GV4S1 or GV5S1-encoded TCR and locates in the intestinal epithelium (i-IEL). Biased expression of particular Vγ regions in specific anatomical sites by the last two sets of γδ T cells presumably reflects a local selection driven by related antigens, but the relatively high combinatorial and junctional diversity of their TCRs suggest that the selecting antigens must be quite heterogeneous or impose fewer structural constraints on their recognition receptors. **See also**: Epithelial Cells: Immunological Aspects; Lymphocytes: Intraepithelial; Thymus

Ontogeny

αβ/γδ lineage split

Although some T-cell subsets, such as the intestinal αβ and γδ intraepithelial T lymphocytes (IELs), develop mainly through an extrathymic differentiation pathway, the thymus remains the main site of differentiation of both αβ and γδ T cells. While these two lineages share common early progenitors, the point at which thymocytes become committed to one or other lineage has not been precisely determined. *In vivo* repopulation and *in vitro* differentiation experiments using early thymocytes (i.e. before acquisition of CD3/TCR complexes) have not led to clear answers, mainly because of the poor definition of the precursor populations studied. A more fruitful way of addressing this issue was to study the TCR rearrangement events occurring in developing αβ and γδ thymocytes. TCR gene rearrangements occur in a sequential order during thymic ontogeny, TCR γ, δ and β loci being rearranged first and TCR α locus a day later. Three models of αβ/γδ lineage separation have been considered: (1) a 'sequential' model, wherein γδ T cells are produced first and αβ T cells develop from precursor cells that have failed to produce functional γ and/or δ gene rearrangements; (2) a 'competitive' model, stating that γ, δ and β gene rearrangements occur simultaneously in uncommitted thymocytes: if γ and δ genes are productively rearranged first, the cell becomes a γδ T cell, if the β gene is productively rearranged first, the cell commits to the αβ lineage and (3) an 'independent lineage' model, stating that αβ and γδ T cells develop through independent pathways (i.e. lineage commitment is independent of the outcome of the rearrangement events occurring in the precursor cell). **See also**: Lymphocyte Development

The last two models are currently favoured: while the competitive model is supported by recent analyses of thymocyte rearrangement status, the independent lineage model fits well with: (1) the existence of silencer elements repressing the transcription of TCR α and γ genes in a lineage-specific fashion; (2) the lack of perturbation of αβ or γδ T-cell development following inactivation of γδ or αβ TCR genes, respectively and (3) the differential cytokine requirements for αβ and γδ T-cell development (e.g. inactivation of the IL-7 gene abrogates γδ and NK-cell development but has little effect on the production of αβ T cells). **See also**: Cells of the Immune System

Programmed rearrangement and expression of TCR V genes

Vγ and Vδ genes are rearranged in a highly ordered fashion during thymic ontogeny. In mice, most fetal thymocytes express nondiversified DV101S1 TCR chains, paired first with GV1S1 + (at day 14–15 of gestation) and then with either GV2S1 + and GV5S1 + chains (at day 16–19 of fetal life). These fetal populations become rapidly undetectable in the thymus after birth but are found in several epithelial sites in adults, such as epidermis and reproductive organ mucosa, thus suggesting an early homing from the fetal thymus to these peripheral tissues. Thymic waves of γδ T cells developing after birth express junctionally diverse TCR chains bearing GV3S1, GV4S1, GV5S1 and GV5S2 regions and Vδ regions distinct from DV101S1. Similarly, in humans there is some evidence for the existence of an early thymic wave of γδ T cells expressing GV2S1/DV102S1 TCR, followed by cells using more upstream V genes. In both species, the V gene rearrangement order follows the genomic position of these elements within the TCR γ and δ loci, the earliest waves using the most proximal VJ elements, the latest waves using the most distal elements. Several other important factors (developmentally regulated expression of the selecting ligands, activation of specific *cis*-acting transcriptional factors) critically contribute to the emergence of these distinct T-cell waves.

Intrathymic selection of γδ T cells

During their intrathymic development, αβ thymocytes go through several 'checkpoints' that allow selective survival and expansion of cells that have successfully completed the previous maturation steps and elimination of the others. There are two main checkpoints. After production of a functional β chain, precursor cells will receive maturation signals from the 'preTCR' (β/pTα) that will lead to their differentiation into CD4/CD8 double-positive (DP) thymocytes. Then DP thymocytes that have produced 'selectable' αβ TCR (i.e. showing enough affinity/avidity for self MHC–peptide complexes) will receive signals leading to their terminal maturation (positive selection), if their TCRs show intermediate avidity for self, or to apoptosis (negative selection) if their TCR are strongly self-reactive. Whether γδ T cells are subjected to such a complex epigenetic control is still debated. While it is now clear that these cells do not proceed through a DP stage and do not receive early maturation signals through pre-TCR-like structures, γδ T cells, like αβ T cells, are subjected to both positive- and negative-selection processes. Indeed, in mice transgenic for γδ TCR directed against polymorphic MHC class Ib molecules, cells bearing transgenic γδ TCR strongly reactive against self MHC class Ib alleles are eliminated or inactivated (i.e. they are negatively selected), and they fail to develop in mice devoid of MHC class Ib molecules (i.e. they need positive selection signals). **See also**: Apoptosis: Molecular Mechanisms; Immune Response: Regulation; Lymphocytes: Antigen-Induced Gene Activation; Transgenic Animals in Immunology

Antigen Recognition

The way γδ T cells recognize antigens was elusive for a while, as both MHC-unrestricted and MHC-restricted γδ cells had been characterized. It is now clear that γδ T cells differ mainly from αβ T cells in their requirements for antigen recognition. It has been demonstrated on theoretical grounds that the antigen-binding loops of the γδ TCR

are more similar to those of Ig than of αβ TCR. Indeed the lengths of CDR3 sequences from antigen recognition receptor chains have been compared, and their distribution was found to be much narrower for αβ TCR than for Ig and γδ TCR. The constrained junctional lengths of TCR α and β CDR3 might reflect recognition of antigens in a restricted cellular context (i.e. MHC molecules), whereas the wide CDR3 length distribution of IgH and TCRδ chains might reflect recognition of a wider set of conformational epitopes on native molecules. The hypothesis that γδ TCR might recognize antigens in an Ig-like mode is now supported by considerable experimental evidence. Structurally unrelated proteins such as gIg, a herpes simplex virus glycoprotein, the murine MHC class II molecules I-Ek or the nonclassical MHC class Ib products T10 and T22 are well-documented examples of antigens recognized by γδ T cells. In these cases, the protein is the actual antigen, i.e. it is directly recognized without any known requirement for processing or presentation. Its recognition is always based on a TCR-mediated interaction with conformational epitopes found in highly heterogeneous experimental systems (e.g. an MHC class Ib molecule, T22 MHC, $Q_{a,b}$ complexed with a glutamic-tyrosine copolymer, a peptidic fragment of tetanus toxoid, heat-shock proteins, etc.). The crystal structure of γδ TCR complexed with MHC-like T22 has provided a first example of such complex, explaining why γδ T cells seem to barely use the large diversity of their TCRγ and TCRδ sequences. Unlike αβ TCR, this γδ TCR did not bind to the top of the MHC-like molecule, but rather hanged off laterally, interacting only through its TCRDδ2-encoded amino acids. Another typical example of nonconventional antigen recognition by γδ T cells is provided by human GV2S1/DV102S1 + T cells, the predominant peripheral blood γδ T-cell subset in human adults. The γδ TCR lymphocytes presents an overall Ig fold resembling to the αβ TCR, except its smaller elbow angle of the V:C domain border. Its highly protruding CDR3γ and CDR3δ loops could enable direct interactions with small ligands such as phosphoantigens (**Figure 1**). **See also**: Antigens; Antigen–Antibody Binding; Antigen Recognition by Lymphocytes; Superantigens

This γδ subset was initially detected by its dual cross-reactivity to mycobacterial pathogens and human B lymphoma. It is now well established that this reflected recognition of the same set of highly unusual antigens are from both kinds of cells. These antigens are low molecular weight compounds with nonpeptide structures (300 Da), either organic pyrophosphates commonly referred to as phosphoantigens, or nonphosphorylated molecules whose bioactivity relates directly to the above phosphoantigens.

Natural phosphoantigens are produced by most live cells, bacteria, eukaryotic pathogens such as Plasmodia, plants and human cells including especially tumours. All these phosphoantigens are endogenous metabolites of cholesterol biosynthesis. In bacteria, algae and some plastids, this metabolism is called the DOXP pathway for involving the deoxy-xylulose-phosphate carbohydrate and yields as intermediate (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate, referred below to as hydroxy-DMAPP (HDMAPP) for reader's convenience. At nanomolar concentrations, HDMAPP is very selectively recognized by GV2S1 /DV102S1 + γδ T cells, and enables their ability to mediate the immune surveillance of microbial infections.

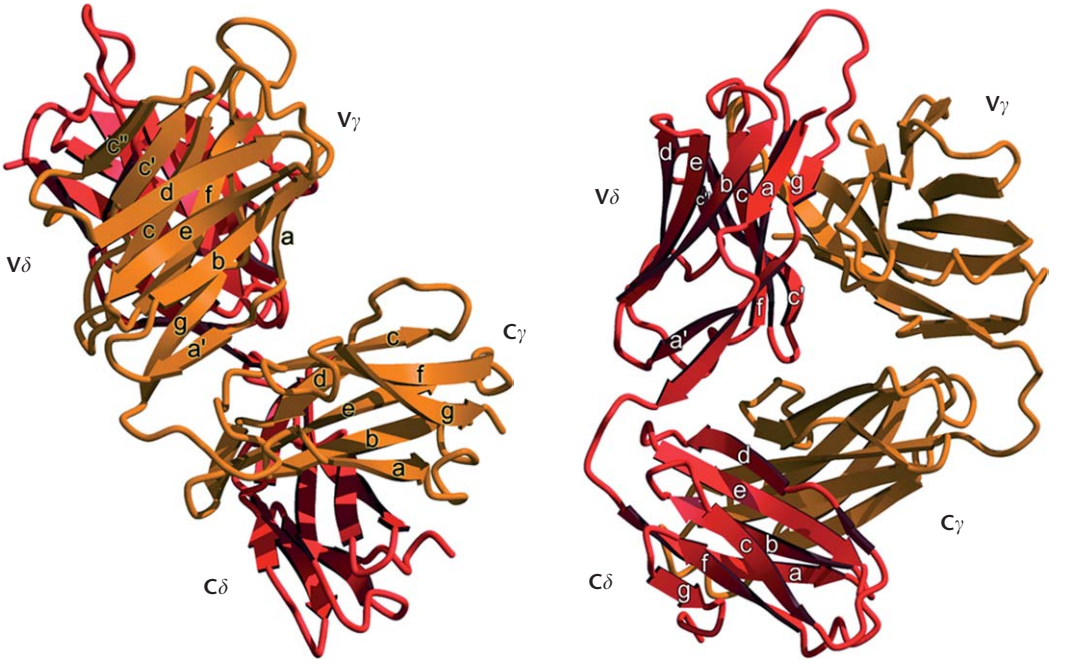

Figure 1 Structure of the human γδ T-cell receptor. Courtesy of D. Garboczi.

In human and most eukaryote cells, by contrast, biosynthesis of cholesterol proceeds by a different metabolic route involving mevalonate. This 'mevalonate pathway' produces two metabolic intermediates, the dimethylallyl-pyrophosphate (DMAPP) – related to the above HDMAPP – and its isomer isopentenyl-pyrophosphate (IPP). Both IPP and DMAPP are also selectively recognized by GV2S1/DV102S1+ γδ T cells, though at one thousand times higher concentrations than microbial HDMAPP. So, because IPP and DMAPP are produced by all our cells, how do our own γδ lymphocytes discriminate normal human cells to ignore from tumours to eliminate? This is simply due to their respective concentrations of phosphoantigens: normal cells only produce subnanomolar concentrations of IPP without bioaccumulation, whereas tumours often show an upregulated mevalonate pathway i.e. able to produce endogenous concentrations of IPP/DMAPP phosphoantigens above the minimal detection threshold. Thus, reactivity to either microbial or tumour-derived phosphoantigens enable the main subset of peripheral γδ lymphocytes to survey for infections and tumours (**Figure 2**).

In addition, reactivity to phosphoantigens is controlled by INMRs expressed by the γδ T cells. This control operates by a counterbalance of the kinases transducing the activating signal from the TCR by intracellular phosphatases triggered by the INMR–MHC class I complex (**Figure 3**).

By this means, normal HLA class I^+ cells deliver negative signalling, which is able to induce the γδ T cell to spare autologous cell, whereas tumour cells with frequently altered expression of HLA class I molecules are not able to deliver these protective negative signalling.

Two other classes of nonpeptide molecules, which are able to selectively activate GV2S1/DV102S1+ T lymphocytes, have also been described. These comprise on the one hand alkylamines such as the microbial isobutylamine or ethylamine from brewed tea, and on the other, a group of aminobisphosphonates such as pamidronate, risedronate and zoledronate, common therapeutic drugs for osteolytic diseases. Although primarily thought to behave like the phosphoantigens depicted above, it is now clear that neither alkylamines and aminobisphosphonates stimulate directly the γδ cells. Rather these two classes of drugs block the mevalonate pathway downstream to IPP, and induce bioaccumulation of endogenous phosphoantigens IPP and DMAPP by treated cells.

Chemical synthesis of various organic phosphoesters gave compounds such as ethyl-pyrophosphate or ribose-phosphate weakly – but selectively – bioactive for human γδ lymphocytes. However, second- and third-generation of chemically synthesized drugs selectively activating GV2S1/DV102S1+ T lymphocytes have arisen, leading to molecules active in the nanomolar to picomolar concentration, such as bromohydrin pyrophosphate (BrHPP). The upscaled production of these novel compounds open unique opportunity to directly assess the potentiality of activated γδ lymphocytes in preventive or therapeutic approaches against infections and tumours.

Although phosphoantigens activate γδ T cells, by an interaction with the TCR yet to be demonstrated, this reactivity is not restricted by polymorphic MHC molecules. Therefore, the use of phosphoantigens is not limited to small subgroups of the human population harbouring the adequate HLA. Consequently, the ubiquitous distribution of phosphoantigens (see above) make them likely responsible for the *in vivo* peripheral expansion of GV2S1/DV102S1+ T cells that occurs during the first years of life.

Function

Given the marked differences between γδ T-cell subsets in terms of tissue distribution, TCR diversity and recognized antigens, γδ T cells cannot be considered as a homogeneous lymphoid population fulfilling a single specialized function. In fact, analysis of mice deficient for either αβ or γδ T cells has revealed different functions played by γδ T cells: an early protective role partly redundant to that of αβ T cells, and a late immunomodulatory function more specifically fulfilled by this lymphoid subset.

The former role is supported by the reactivity of γδ T cells against native epitopes carried by widely distributed and highly conserved bacterial ligands (such as the bacterial phosphoantigens activating human γδ T cells) and their ability to rapidly produce proinflammatory cytokines (interferon γ, tumour necrosis factor α, interleukin 2, etc.) upon recognition of these antigens. In support of the latter function, secondary αβ T-cell responses against various infectious agents (e.g. listeria, protozoa) are exacerbated in mice lacking γδ T cells, when compared to wild-type mice. Furthermore, several γδ T-cell subsets (e.g. the murine GV5S1/DV104S1+ or the human GV2S1/DV102S1+ cells) were shown to modulate αβ T-cell function *in vitro* and *in vivo*. In addition, γδ T cells crosstalk with dendritic cells in the priming of adaptive immune responses, leading to reciprocal regulation of maturation. Activation of human peripheral γδ T cells might confer these cells some antigen-presenting functions. This homeostatic role for γδ T cells probably extends beyond the immune system, as murine intraepidermal γδ T cells are activated by stressed keratinocytes and can secrete keratinocyte growth factor (KGF), thus suggesting their implication in epithelial homeostasis. A similar role is suspected for human and murine intestinal γδ IELs. In mice, these cells largely predominate in the intestinal epithelium of mice maintained in a germ-free environment but are rapidly diluted out by αβ T cells when mice are exposed to bacteria. Since intestinal γδ IEL also produce KGF, these results would support their implication in the control of intestinal epithelium integrity through recognition of conserved self epithelial antigens. Along this line, some human intestinal γδ IELs were recently shown to recognize weakly polymorphic MHC class Ib products called MICA and MICB, which are specifically expressed on activated and transformed epithelial cells. Similar regulatory functions of γδ T cells have also been demonstrated in allergy and in oral tolerance, although their stimulatory or modulatory role depends on the

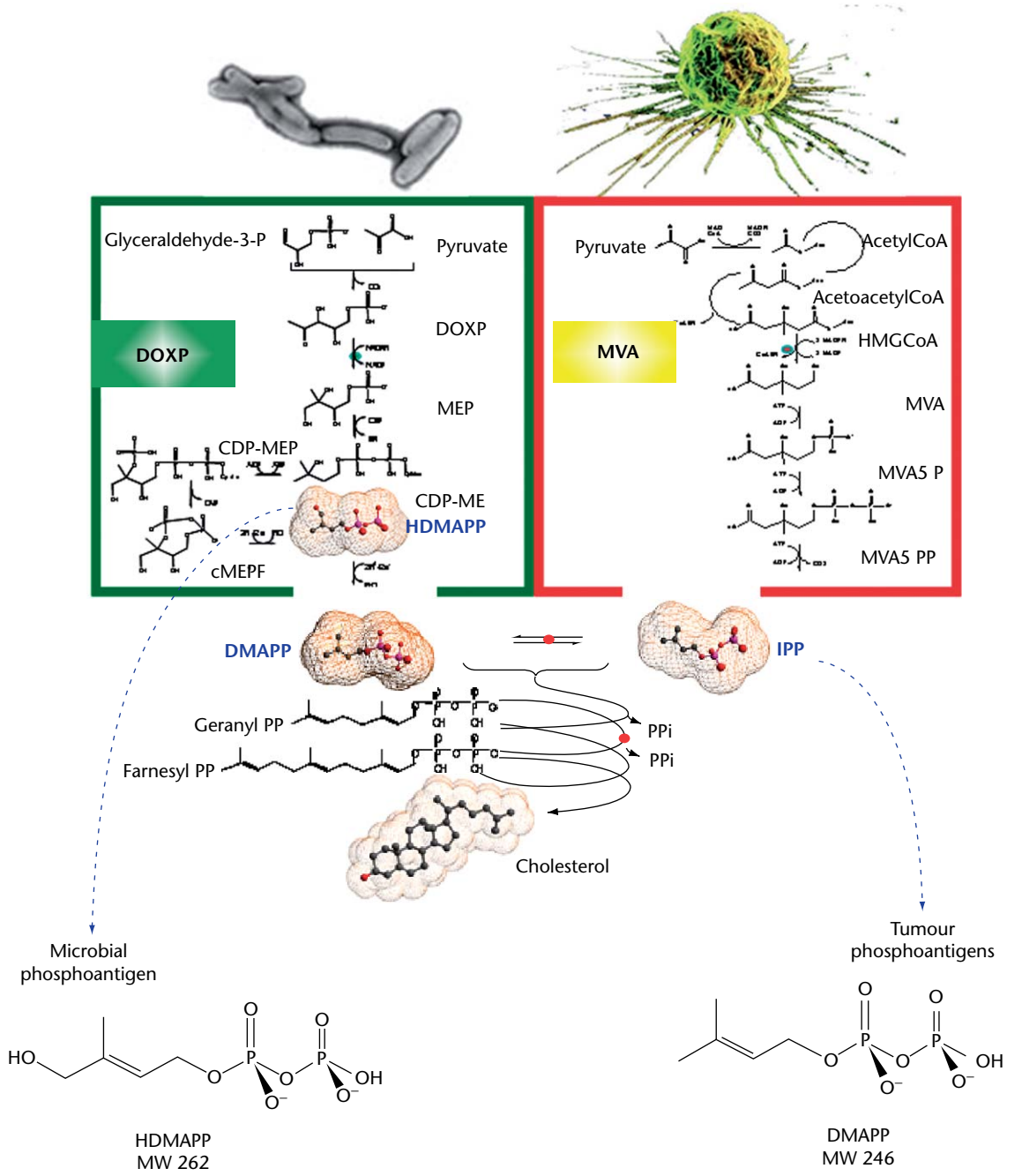

Figure 2 Biosynthesis of nonpeptide phosphoantigens recognized by human γδ T lymphocytes. Left, microbial pathogens, e.g. *Mycobacterium tuberculosis*, the agent of human TB produces the HDMAPP phosphoantigen to make cholesterol; right, in human cells, cholesterol biosynthesis proceeds by the mevalonate pathway and produces IPP and DMAPP phosphoantigens.

experimental conditions. **See also**: Cytokines; Epitopes; Epithelial Cells: Immunological Aspects; Immunological Tolerance: Mechanisms; T-lymphocyte Activation

Given the similarities existing between activated and transformed cells, it is not surprising that γδ subsets recognizing the former (i.e. taking part in homeostatic processes) also recognize the latter, and thus play a role in tumour surveillance. In this regard, mice transgenic for a γδ TCR presumably directed against a self molecule expressed by activated αβ T cells developed an increased resistance to various acute leukaemias. Similarly in humans, the major GV2S1DV102S1 + T-cell subset, which is stimulated upon an MHC-unrestricted recognition of phosphoantigens, kills activated T cells upon masking of its inhibitory

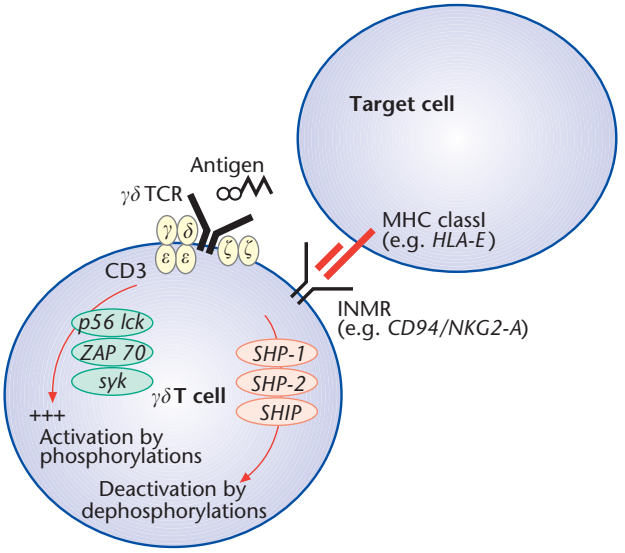

Figure 3 Stimulation of a human γδ T lymphocyte by the MHC-unrestricted recognition of a small nonpeptidic antigen. Its TCR-mediated activation threshold is balanced by inhibitory signals triggered by the interaction of INMR with MHC class I on target cells. Zap, ζ-associated protein of 70 KDa.

MHC-specific receptors, and also displays strong cytolytic activity against a wide array of lymphoid tumours. For example, GV2S1DV102S1+ T-lymphocytes engage immunological synapses with anaplastic lymphoma target cells and within minutes, are able to kill them by the release of intracellular granules of perforin (**Figure 4**). **See also**: Leukaemias and Lymphomas; Tumour Immunology

Hence, this subset displays important anticancer function that could be boosted upon activation by synthetic phosphoantigens. As the potential for cancer immunotherapy of these immunostimulatory drugs is now widely accepted, several clinical trials are currently ongoing to harness this new family of therapeutic cells in lymphoma and solid tumours.

Conclusion

Gamma delta T cells probably play two important roles that complement those fulfilled by αβ T cells. During primary responses, these cells can rapidly mount responses against a small set of phylogenetically conserved but structurally diverse ligands. They may thus represent a first line of defence against external insults and probably act as an efficient link between adaptive and nonadaptive immunity. During later stages of the immune response, γδ T cells

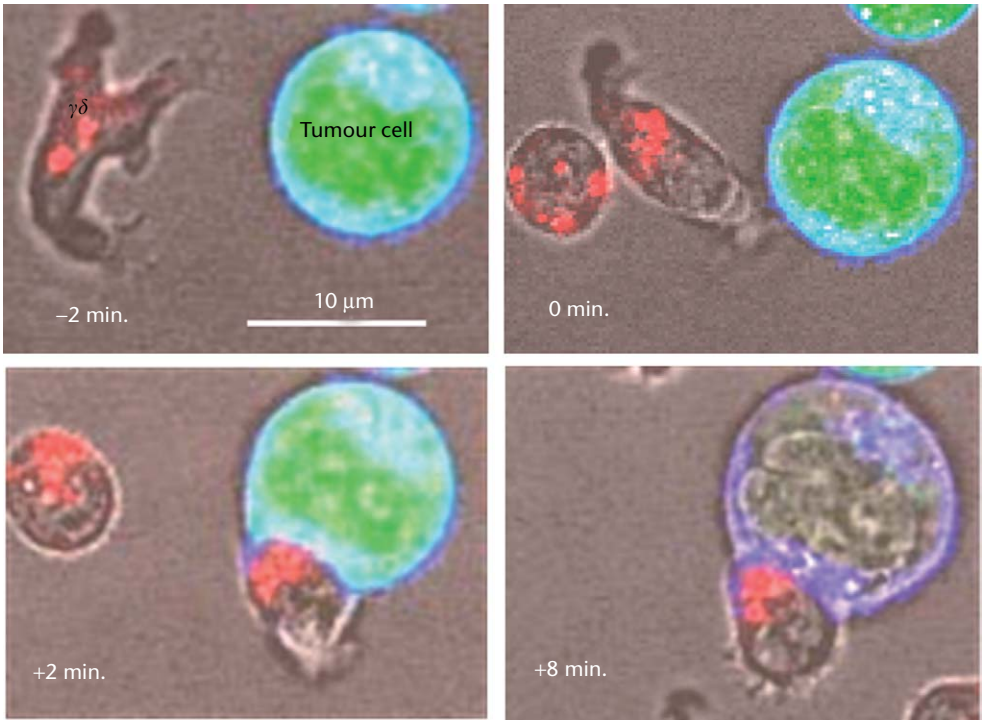

Figure 4 A human γδ T lymphocyte rapidly scans the surface of an anaplastic lymphoma cell target (green cytoplasm and blue membranes) for stimulating and inhibitory signals. Once fully activated, the γδ T lymphocyte kills this target by secreting its cytolytic perforin granules (stained red). The green fluorochrome (calcein AM) is a viability cytoplasmic stain, whose loss indicates cell death. For easier views, the target cell membrane was labelled with a blue fluorochrome.

probably take part in the control of αβ T-cell activation, through recognition of self ligands upregulated during immune activation. Additionally, the peculiar tissue distribution of γδ T cells, their trophic function for epithelia and their ability to recognize stressed or activated epithelial cells suggest an important role in nonimmune homeostatic processes. Ongoing developments of innovative cancer immunotherapies will put in practise the antitumoral potential of human γδ lymphocytes, activated by a new class of compounds such as the synthetic phosphoantigen analogues.

References

Haas W, Pereira P and Tonegawa S (1993) Gamma/delta cells. *Annual Review of Immunology* **11**: 637–685.

Lefranc MP (1990) Organization of the human T-cell receptor genes. *European Cytokine Network* **1**(3): 121–130.

Raulet DH (1989) The structure, function, and molecular genetics of the gamma/delta T cell receptor. *Annual Review of Immunology* **7**: 175–207.

Yoshikai Y, Toyonaga B, Koga Y *et al*. (1987) Repertoire of the human T cell gamma genes: high frequency of nonfunctional transcripts in thymus and mature T cells. *European Journal of Immunology* **17**(1): 119–126.

Further Reading

Allison TJ and Garboczi DN (2002) Structure of gammadelta T cell receptors and their recognition of non-peptide antigens. *Molecular Immunology* **38**: 1051–1061.

Blattman JN and Greenberg PD (2004) Cancer immunotherapy: a treatment for the masses. *Science* **305**: 200–205.

Bonneville M and Fournie JJ (2005) Sensing cell stress and transformation through Vgamma9Vdelta2 T cell-mediated recognition of the isoprenoid pathway metabolites. *Microbes and Infection* **7**: 503–509.

Fournie JJ, Bonneville M and Romagne F (2005) Mechanisms of action of non peptide antigens activating human Vγ9Vδ2 T cells and their potential use for immunointervention. *Current Medicinal Chemistry_Anti-Inflammatory and Anti-Allergy Agents* **4**: 161–168.

Hayday AC (2000) [gamma][delta] cells: a right time and a right place for a conserved third way of protection. *Annual Review of Immunology* **18**: 975–1026.

Kabelitz D, Wesch D, Pitters E and Zoller M (2004) Potential of human gammadelta T lymphocytes for immunotherapy of cancer. *International Journal of Cancer* **112**: 727–732.

Kunzmann V and Wilhelm M (2005) Anti-lymphoma effect of gammadelta T cells. *Leukemia & Lymphoma* **46**: 671–680.

Sanders JM, Ghosh S, Chan JM *et al*. (2004) Quantitative structure–activity relationships for gammadelta T cell activation by bisphosphonates. *Journal of Medicinal Chemistry* **47**: 375–384.

Zitvogel L, Tesniere A and Kroemer G (2006) Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nature Reviews Immunology* **6**: 715–727.