Human Vγ9Vδ2 T cells: promising new leads for immunotherapy of infections and tumors

Marc Bonneville and Emmanuel Scotet

Vγ9Vδ2 T cells, a major human peripheral γδ T-cell subset, react *in vitro* against a wide array of microbial agents and tumor cells. This broad reactivity pattern is conferred by non-peptidic phosphorylated isoprenoid pathway metabolites, referred to as phosphoantigens, which are able to specifically activate this γδ T-cell subset in a T-cell receptor dependent fashion. Recent studies provide new insights into the mode of action of phosphoantigens on Vγ9Vδ2 T cells and might explain how their recognition can allow detection of infected or altered self by the immune system. The broad antimicrobial and antitumoral reactivity of Vγ9Vδ2 T cells, their ability to produce inflammatory cytokines involved in protective immunity against intracellular pathogens and tumors, and their strong cytolytic and bactericidal activities suggest a direct involvement in immune control of cancers and infections. These observations have recently aided development of novel immunotherapeutic approaches aimed at Vγ9Vδ2 T-cell activation, which have already yielded encouraging results.

Addresses
Institut National de la Sante et de la Recherche Medicale, Unite 601, Institut de Biologie, 9 quai Moncousu, 44093 Nantes cedex 01, France

Corresponding author: Bonneville, Marc (bonnevil@nantes.inserm.fr)

Current Opinion in Immunology 2006, **18**:539–546

This review comes from a themed issue on
Innate lymphocytes
Edited by Albert Bendelac

Available online 25th July 2006

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2006.07.002

Introduction

Since the fortuitous discovery of γδ T cells about two decades ago, more than 5000 articles have been written that address the biology of these lymphocytes in animal and human models. These studies have highlighted the marked molecular, phenotypic and functional heterogeneity of γδ T cells [1]. In particular, they have revealed the striking tissue-dependent restriction of the γδ T-cell receptor (TCR) repertoire in rodents, which results, in some cases, in the expression of the same 'invariant' TCRs by γδ T cells that reside in particular tissues, such as epidermis or reproductive organ mucosa [2]. These observations suggest that murine γδ T cells in different body locations have distinct functions, which is in accordance with reports that implicate these lymphocytes in protective immunity against pathogens and tumors, immunoregulation or epithelial homeostasy. However, the structural basis for such a functional specialization is still ill-defined because we know little about the fine antigen (Ag) specificity of γδ T cells. Besides a few examples of recognition of native MHC or MHC-like molecules or of native viral components by a tiny fraction of γδ T cells derived from peripheral lymphoid organs [3], the Ag specificity of most of them, including rodent intraepithelial subsets that express invariant γδ TCRs, remains undefined.

In humans, most of our knowledge about the specificity and biological role of γδ T cells is derived from analysis of a major peripheral subset referred to as Vγ9Vδ2 T cells, the features of which will be reviewed here. A particular emphasis will be given to recent results that have provided new hints about the mode of activation of these lymphocytes, their implication in immune responses against infectious agents and tumors, and their manipulation for immunotherapeutic purposes.

Early maturation of Vγ9Vδ2 lymphocytes

Like their murine counterparts, human γδ T cells show biased usage of particular TCR V regions that differs between tissue locations [4,5]. Whereas most thymic or splenic γδ T cells express Vδ1 or Vδ3 TCRs, the majority of γδ peripheral blood lymphocytes (PBLs) in human adults express TCRs comprising Vδ2 and Vγ9 regions (also referred to as Vδ2 and Vγ2 in some reports). The high frequency of peripheral Vγ9Vδ2 T cells, which make up to several percent of $CD3^+$ PBLs in adults, is probably the consequence of their postnatal peripheral expansion [4]. This process is paralleled by early acquisition of memory markers, because, unlike other γδ or αβ subsets, almost all Vγ9Vδ2 PBLs already display memory features in two-year old infants [6]. Although repeated stimulation by environmental Ags could explain the rapid immunological maturation of postnatal Vγ9Vδ2 PBLs, the common occurrence of Vγ9Vδ2 T cells that carry memory markers in cord blood (on average 40%) suggests their recurrent stimulation by endogenous ligands upregulated during early developmental stages.

Despite significant sequence diversity at their V(D)J joints, most Vγ9Vδ2 PBLs display recurrent TCR junctional features that are not found on most Vγ9Vδ2 thymocytes [7]. This suggests their peripheral selection by structurally related Ag. Accordingly, PBL-derived Vγ9Vδ2 T-cell clones show similar reactivity patterns against infected and tumor cells, and are activated in a

TCR-dependent fashion by the same restricted set of non-peptidic compounds (see below). Therefore, Vγ9Vδ2 T cells can be considered as a monospecific lymphoid subset.

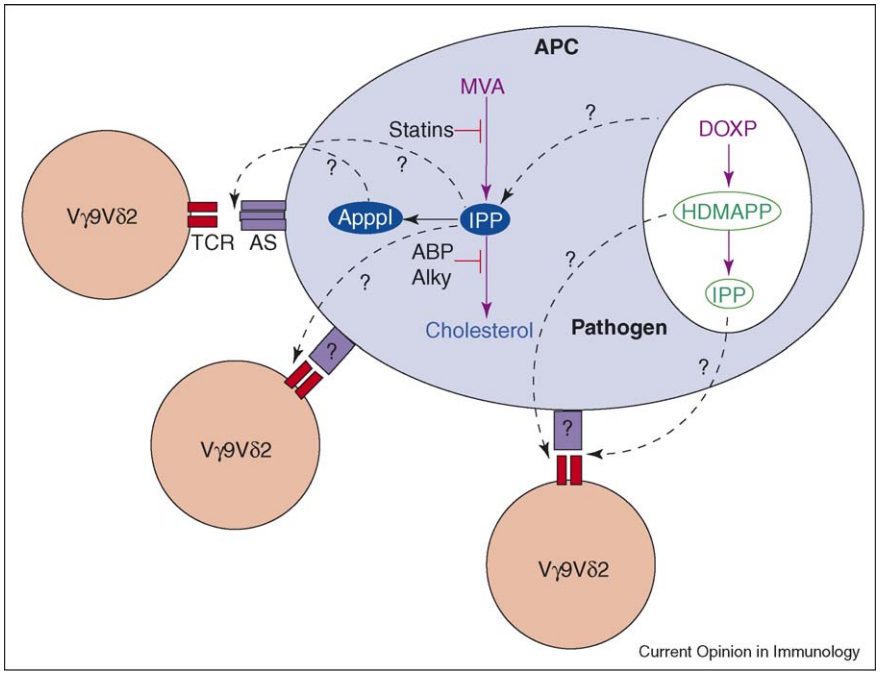
Vγ9Vδ2 recognition of microbial and tumor-derived isoprenoid metabolites

Vγ9Vδ2 T cells are specifically activated by small non-peptidic phosphorylated compounds — also referred to as phosphoantigens (phosphoAgs) [4]. In all instances, these molecules are metabolites of isoprenoid biosynthetic pathways (Figure 1).

The most potent Vγ9Vδ2 agonists are produced through the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway, which is found in several microorganisms [8,9]. One such metabolite, called hydroxy-dimethyl-allyl-pyrophosphate or hydroxy-methyl-butyl-pyrophosphate, activates Vγ9Vδ2 T cells at 0.1 nM concentrations [8]. By contrast, the Vγ9Vδ2 bioactivity of metabolites produced through the mevalonate pathway used by mammalian cells, such as isopentenyl pyrophosphonate (IPP), is about 10 000-fold lower [10]. The high specific bioactivity of microbial phosphoAgs might allow Vγ9Vδ2 T cells to efficiently and sensitively detect target cells infected by even a single mycobacteria [11•] (Figure 2). Moreover, efficient discrimination of normal versus tumor cells by this γδ subset is ensured through activation by IPP, the production of which is increased upon cell transformation [12]. Upregulation of stress ligands (such as NKG2D ligands) on infected and transformed cells might further enhance specific recognition of altered-self by Vγ9Vδ2 T cells, in line with recent studies that reported the important role played by NKG2D receptors during target cell killing by Vγ9Vδ2 T cells [13–16].

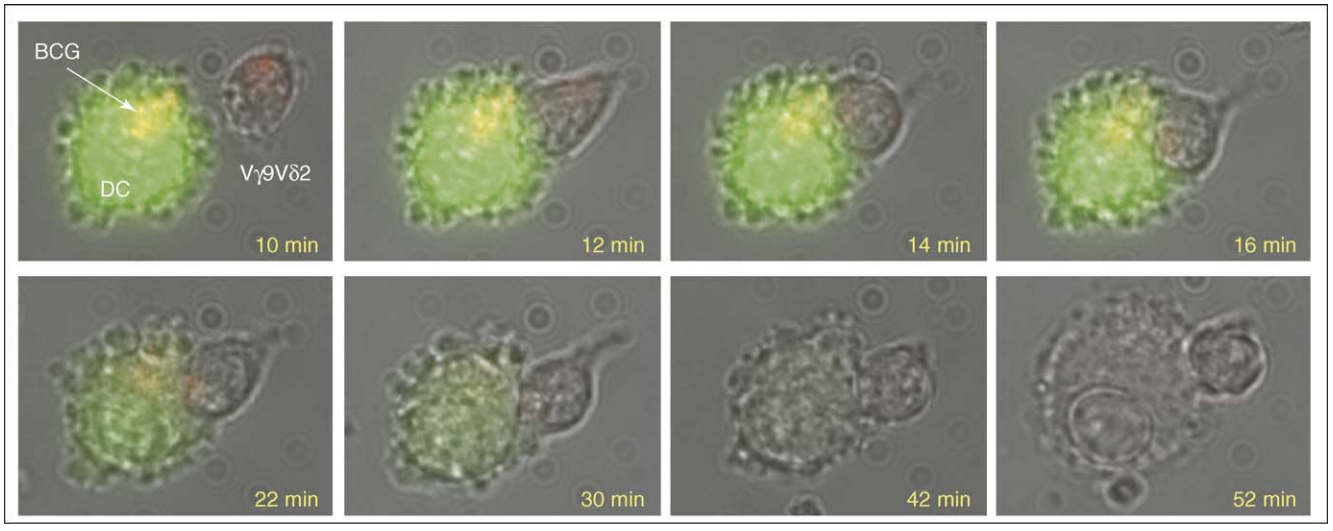
In addition to phosphoAg, pharmacological compounds that inhibit the mevalonate pathway such as statins and aminobisphosphonates (ABPs) can modulate target cell recognition by Vγ9Vδ2 cells by down- and up-regulating intracellular IPP levels, respectively [4]. The ability of some nonphosphorylated compounds, such as alkylamines [17], to stimulate Vγ9Vδ2 lymphocytes has been difficult to reconcile with the above findings. Recent observations indicate that, like ABPs, alkylamines are not recognized *per se* but instead inhibit the mevalonate pathway downstream of IPP biosynthesis, thus leading to intracellular accumulation of this phosphoAg [18•]. Therefore, it appears that, in all instances, Vγ9Vδ2 T cells are activated by pyrophosphomonoesters.

Figure 1



Contribution of DOXP versus mevalonate (MVA) metabolites to Vγ9Vδ2 T-cell activation. In mammalian cells, IPP is a metabolite produced through the isoprenoid MVA pathway that leads to cholesterol production. Pharmacological agents can block either upstream (statins) or downstream (ABP, alkylamines) MVA pathways leading, respectively, to decreased or increased intracellular IPP levels. Endogenous IPP accumulation is also observed in diverse tumor cells. This metabolite could then be directly presented at the cell surface either by peroxysomal or mitochondrial enzymes translocated at the cell surface, such as AS, or by a presentation molecule that has not yet been defined. IPP metabolites can be converted into ApppI — a recently described ATP analog — which could then be processed and presented at the cell surface. In pathogen-infected APCs (e.g. mycobacterial infection), bacterial 4-hydroxy-3-dimethylallyl pyrophosphate (HDMAPP) or IPP metabolites produced through the DOXP pathway could be directly presented and/or contribute to upregulate the cellular MVA pathway.

Figure 2



Vγ9Vδ2 T lymphocytes can kill *in vitro* cells infected by mycobacteria. These fluorescence images show an interaction between a single cytotoxic Vγ9Vδ2 T cell (clone G115) and a *M. bovis* BCG-infected immature dendritic cell (DC). DCs were first infected for two hours with enhanced green fluorescent protein (EGFP)–BCG, and were then washed and loaded with calcein (green), which was used as a viability marker in this experiment. T lymphocytes were loaded with Lysotracker Red (red), which labels lytic granules. Time after addition of T cells to infected DCs is indicated in each box. Vγ9Vδ2 cytotoxic lymphocyte rapidly forms a conjugate with a BCG-infected DC (10–12 min). Lytic granules (red) are reoriented towards infected DC and are relocalized close to the immune synapse (12–16 min). BCG-infected DC is killed by Vγ9Vδ2 lymphocyte, as visualized by calcein leakage and morphological changes (22–52 min).

Several issues regarding the respective contribution of DOXP versus mevalonate metabolites to Vγ9Vδ2 activation and their mode of action remain open. Statins, which block the mevalonate but not the DOXP pathway, significantly inhibit *in vitro* recognition of infected cells by Vγ9Vδ2 T cells (MB and ES, unpublished). Although preliminary, these data indicate that, despite its low bioactivity, IPP might contribute to Vγ9Vδ2 activation in an infectious context, possibly as a consequence of an upregulation of the mevalonate pathway upon infection and/or phagocytosis. According to this hypothesis, the broad antiviral reactivity of Vγ9Vδ2 T cells [19•] could be accounted for by upregulation of cellular IPP. Together, these results indicate that IPP is a sensor of cell stress, not only in a tumor but also in an infectious context.

Mode of Vγ9Vδ2 activation by phosphoantigens

Although phosphoAg-mediated activation of Vγ9Vδ2 T cells clearly involves TCRs, all attempts to demonstrate cognate interactions between Vγ9Vδ2 TCRs and phosphoAgs in acellular systems have failed to date [4]. Because activation of Vγ9Vδ2 T cells requires cell-to-cell contact [20], these results suggest either that phosphoAgs induce structural modification of surface receptors recognized in turn by the Vγ9Vδ2 TCRs or that phosphoAgs are presented by surface molecules that are undefined to date. Although mevalonate pathway enzymes that interact with IPP are normally located in peroxisomes, some of these enzymes might interact with chaperones involved in surface translocation of intracellular proteins [21]. Therefore, they might directly present phosphoAgs to the TCR. Alternatively, IPP could be presented by surface receptors unrelated to the mevalonate pathway. In this regard, Vγ9Vδ2 T cells were shown recently to recognize a complex formed between apolipoprotein A1 and ATP synthase (AS) — a mitochondrial enzyme that is translocated to the surface of normal hepatocytes and some tumor cell lines — in a TCR-dependent fashion [22••]. Direct activation of Vγ9Vδ2 T cells by AS-coated beads suggests that apolipoprotein A1 plays a dispensable role in this process, although the latter might enhance the interaction between Vγ9Vδ2 TCR and AS.

The biological relevance of AS recognition by Vγ9Vδ2 lymphocytes and the possible implication of this enzyme in phosphoAg presentation are still unclear. The mechanisms that underlie translocation of AS to the plasma membrane are unknown but could be linked to accidental fusion events between the mitochondrial and plasma membranes, which might occur in actively proliferating and/or transformed cells. Accordingly, surface AS is detected on proliferating but not on resting keratinocytes [23]. Owing to its extensive conservation from bacteria to mammals, AS could also represent a cue for activation of Vγ9Vδ2 T cells that is upregulated in diverse infectious contexts. In this regard, the AS β subunit is among the few microbial proteins that can be detected in

mycobacteria-infected macrophages early after infection [24]. Surface AS is also expressed on endothelial cells, in which it inhibits cell proliferation after engagement by angiostatin [25]. Therefore, AS recognition might allow not only direct activation of Vγ9Vδ2 T cells by infected and/or transformed cells but also immune regulation of neo-angiogenesis. Although direct interactions between AS and phosphoAg have not been reported to date, a recent study suggests a link between AS and phosphoAg recognition by Vγ9Vδ2 T cells. IPP accumulation in ABP-treated cells is associated with appearance of ApppI — a novel ATP analog that results from covalent bonding between AMP and IPP [26]. Such a compound, which is reminiscent of several Vγ9Vδ2-stimulating nucleotidic mycobacterial phosphoAgs and was previously referred to as TubAg3 and TubAg4 [27], is able to induce cell apoptosis through blockade of mitochondrial ATP/ADP translocase. Similarly, ApppI might interact with ATP synthase, thus raising the possibility that Vγ9Vδ2 T cells actually recognize such a complex. Although highly speculative, such a hypothesis could be tested by analysis of ApppI binding to purified AS and its effect on Vγ9Vδ2 T-cell activation.

In vitro Vγ9Vδ2 T cell responses against infections and tumors

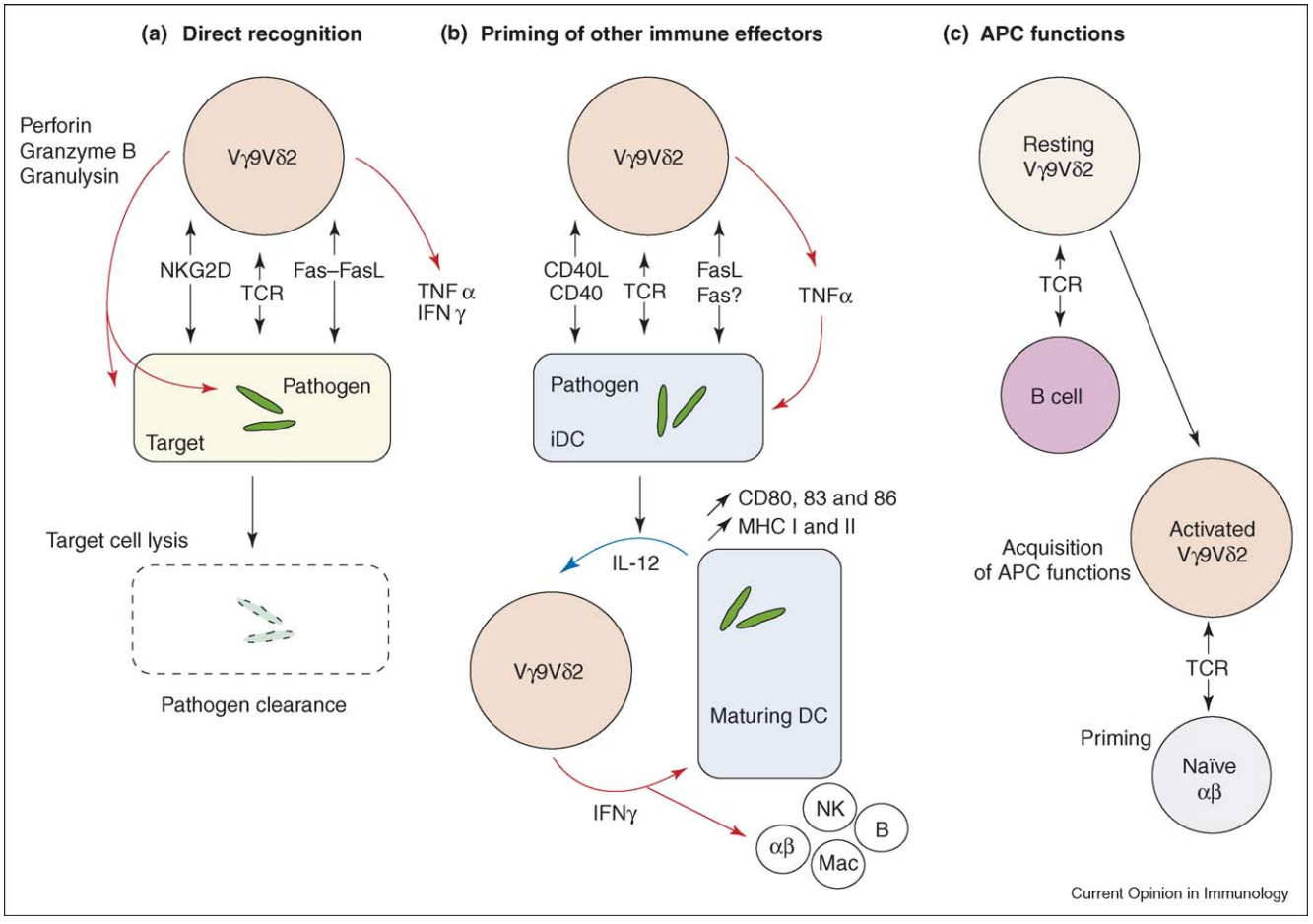
Implication of Vγ9Vδ2 T cells in anti-infectious immunity and tumor immunosurveillance is supported by both *in vivo* and *in vitro* observations. *In vivo*, peripheral blood and/or intralesional Vγ9Vδ2 T cells are expanded in patients infected by a wide array of microbial agents and in those that carry hemopoietic and solid tumors [4]. Moreover, Vγ9Vδ2 T cells kill *in vitro* cells infected by bacteria, protozoa and viruses, as well as by a variety of tumor cell lines [4] (Figure 2). Because most peripheral Vγ9Vδ2 T cells show a memory and pre-activated status [6] and express receptors for inflammatory chemokines such as CCR5 [28,29] they can be rapidly recruited to and locally activated in inflamed tissues in the course of infection or oncogenesis. In these, Vγ9Vδ2 T cells might contribute to the early stage of immune protection through at least three possible mechanisms (Figure 3).

Owing to their strong lytic activity and their ability to release pro-inflammatory cytokines and antibacterial compounds [30], Vγ9Vδ2 T cells can directly mediate elimination of infected and tumor cells and pathogen clearance (Figures 2 and 3a). Vγ9Vδ2 T cells might also contribute to immature dendritic cell (iDC) activation and subsequent priming of conventional Th1 responses (Figure 3b). Like invariant natural killer T cells (iNKT cells), Vγ9Vδ2 lymphocytes can induce full activation of iDCs infected by pathogens unable to promote efficient iDC maturation, such as mycobacteria or α-proteobacteria. In particular, iDCs infected with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) show accelerated and full maturation into IL-12-producing cells when incubated with Vγ9Vδ2 lymphocytes [11•]. However, Vγ9Vδ2 T cells are unable to produce proinflammatory cytokines when incubated with iDCs alone, even in the presence of IL-12 (MB and ES, unpublished). This indicates that, unlike iNKT or NK cells [31,32], this γδ subset shows no basal autoreactivity against DCs and, therefore, might not contribute to priming of immune responses directed against pathogens that lack Vγ9Vδ2 agonists. As for other 'innate-like' lymphocytes, Vγ9Vδ2 T cell mediated iDC activation presumably occurs in two steps: an initial upregulation of costimulatory and MHC I and II receptors is induced upon CD40L engagement and exposure to TNFα produced by activated Vγ9Vδ2 T cells, then IL-12 production is boosted through a positive feedback loop that involves T cell derived IFNγ [33]. In light of a recent study describing Fas-mediated iDC activation by Vδ1 T cells [34•], Vγ9Vδ2 T cells might also activate iDC through Fas engagement, because they show strong upregulation of both FasL and CD40L mRNA upon phosphoAg stimulation [35].

Finally, a recent report from B Moser and co-workers [36•] suggests a new and unexpected implication of Vγ9Vδ2 T cells in naïve T-cell priming, through their ability to behave like professional antigen-presenting cells (APCs; Figure 3c). Unlike αβ T cells, Vγ9Vδ2 PBLs acquire several attributes of APCs, such as the expression of CD40 and costimulatory molecules, upon short-term *in vitro* antigenic stimulation. Accordingly, activated Vγ9Vδ2 T cells can promote *in vitro* proliferation of superantigen-stimulated naïve αβ T cells. Because Vγ9Vδ2 lymphocytes transiently upregulate CCR7 upon Ag activation [29], this would endow them with the capacity to migrate to lymph nodes where they could fulfill these APC functions. However, the above features might not be restricted to Vγ9Vδ2 T cells, but instead reflect a peculiar functional and/or memory status (with respect to CCR7 upregulation) or result from unusual *in vitro* T-cell stimulation conditions. Feeder B cells were required to get optimal acquisition of professional APC characteristics by Vγ9Vδ2 T cells (B Moser, personal communication). Therefore, the possibility that B cell derived costimulatory factors are primarily responsible for this phenomenon has not been formally ruled out. Moreover, the limited pinocytic and phagocytic activity of Vγ9Vδ2 lymphocytes and their inability to produce IL-12 might not allow them to efficiently prime conventional Th1 cells directed against processed peptidic Ag. These various issues will certainly be addressed in future.

Whereas Vγ9Vδ2 T cells are classically considered as proinflammatory lymphocytes that are able to produce large amounts of TNFα and IFNγ [4], some Vγ9Vδ2 subsets show different functional profiles, possibly reflecting their involvement in the regulation of a broader array of immune and non-immune processes. Tonsillar

Figure 3



Multiple contributions of Vγ9Vδ2 T lymphocytes to protective immunity against infections and tumors. **(a)** Direct recognition of tumor or infected target cell. Following TCR engagement, Vγ9Vδ2 T cells release rapidly large amounts of inflammatory cytokines (TNFα and IFNγ) and lytic mediators (perforin, granzyme B and granulysin), leading to the destruction of target cells and internalized pathogens such as mycobacteria. **(b)** Priming of other immune effectors. Vγ9Vδ2 T cells are activated by pathogen-infected DCs and promote accelerated and complete DC maturation through strong release of TNFα and CD40 engagement. Maturing DCs release significant levels of IL-12, which amplifies IFNγ secretion by activated T lymphocytes. Released cytokines, as well as fully matured DCs, can then contribute to the priming of innate and adaptive immune effectors subsequently involved in the clearance of pathogens. **(c)** APC functions. Upon short-term antigenic stimulation, Vγ9Vδ2 T cells acquire several attributes of APCs (CD40, MHC I and II, and costimulatory molecules) and can promote the priming of naïve conventional αβ T cells. B cells might be required to get optimal induction of those APC features.

Vγ9Vδ2 T cells display cytokine and chemokine–chemokine receptor profiles similar to those of the so-called T follicular helper subsets, which have been implicated in the formation of germinal centers ([29] and F Dieli, personal communication). Recent observations suggest that such T follicular helper-like Vγ9Vδ2 T cells are induced *in vitro* upon Ag stimulation in the presence of IL-21 (Vermijlen D *et al.*: Gene expression profiling of unconventional T cells provides novel insights into a human γδ T cell clinical trial [Abstract 36]. 2^{d} γδ T cell conference, San Diego CA, March 2006). It is also possible to polarize *in vitro* Vγ9Vδ2 T cells towards IL-4-producing 'Th0' cells [37]. *In vivo* Th0 polarization of Vγ9Vδ2 PBLs seems to correlate with development of active tuberculosis in exposed healthcare workers, suggesting its relevance as a prognostic marker of immune protection against mycobacterial infections [38]. Finally, recent studies have described production of several factors involved in epithelial regeneration, such as keratinocyte growth factor, fibroblast growth factor 9 and matrix metalloproteinase 7, by Ag-stimulated Vγ9Vδ2 T cells [39,40]. Hence, Vγ9Vδ2 T cells, which are also found within the intestinal epithelium, might contribute to epithelial homeostasis, like murine intraepithelial γδ subsets [41]. Additional *in vivo* studies will be required to validate the biological relevance of these observations. In any case, they illustrate the diversity of Vγ9Vδ2 effector functions, the induction of which might tightly depend on the modalities of Ag stimulation and the tissue microenvironment.

In vivo Vγ9Vδ2 T cell responses in SCID/Hu models

The lack of murine counterparts of Vγ9Vδ2 T cells has dramatically hampered assessment of their *in vivo* role. Nevertheless, the involvement of Vγ9Vδ2 T cells in the immune control of some bacterial infections and solid tumors is suggested by analysis of severe combined immunodeficiency (SCID) mice reconstituted with human PBLs or with purified Vγ9Vδ2 T cells. It has been reported recently that adoptively transferred Vγ9Vδ2 T cells in SCID mice have anti-melanoma activity [42,43]. However, Vγ9Vδ2 cells might not be as efficient as Vδ1 T cells or NK cells in clearing tumors upon systemic injection, possibly owing to expression of inappropriate homing receptors [43]. By contrast, direct *in vivo* activation of Vγ9Vδ2 PBLs by synthetic Vγ9Vδ2 agonists and IL-2 in a SCID/HuPBL model leads to massive infiltration of transplanted renal tumors by *in vivo* expanded Vγ9Vδ2 T cells, followed by efficient tumor clearance (J Chargui *et al.*: Phosphostim-activated Vγ9Vδ2 T cells induce anti-tumoral immunity *in vivo* against renal cell carcinoma [abstract 69]. 2^{d} γδ conference, San Diego CA, March 2006). This indicates that *ad hoc* tumor homing receptors can be induced on this γδ subset upon *in vivo* Ag stimulation.

Vγ9Vδ2 T cell-based cancer immunotherapy

The high frequency of Vγ9Vδ2 lymphocytes in most individuals, their reactivity towards small conserved non-peptidic compounds amenable to *in vitro* synthesis, the diversity of their effector functions, and their broad reactivity against infected and tumor cells make them promising targets for immunotherapy. Several GMP-grade compounds able to stimulate Vγ9Vδ2 T cells are readily available for such purposes.

ABPs have already been used for several years to treat bone resorption associated with various pathologies (e.g. osteoporosis, multiple myeloma, etc.). Moreover, large-scale synthesis of clinical-grade Vγ9Vδ2 agonists identical or related to natural phosphoAgs has been achieved recently by some private companies [44••]. Such compounds are now used for clinical and pre-clinical studies in patients and in non-human primates, which all possess close homologues of human Vγ9Vδ2 lymphocytes. To date, adoptive transfer of several billions of *in vitro* expanded autologous Vγ9Vδ2 T cells in renal carcinoma patients has shown no or limited toxicity in phase I trials (J Bennouna, personal communication).

The next goals will be to design clinical-grade T-cell labelling protocols to monitor tumor homing of injected Vγ9Vδ2 T cells, and to demonstrate their therapeutic efficacy in phase II trials. Several clinical trials aiming at direct *in vivo* activation of Vγ9Vδ2 T cells in cancer patients were recently completed or are ongoing. Transient systemic increase of TNFα and IFNγ serum levels — two cytokines that have known antitumor and/or antimicrobial activity — was observed within a few hours after intravenous injection of ABP or phosphoAg in primates [44••,45] and patients (J Bennouna *et al.*: Phase I clinical trial of Bromohydrin Pyrophosphate, BrHPP [Phosphostim], a Vγ9Vδ2 T lymphocytes agonist in combination with low dose Interleukin 2 in patients with solid tumors [Abstract 72]. 2^{d} γδ conference, San Diego CA, March 2006). However, such treatments failed to induce *in vivo* expansion of Vγ9Vδ2 PBLs in most individuals. In stark contrast, co-administration of both Vγ9Vδ2 agonists and recombinant IL-2 led to a significant expansion of Vγ9Vδ2 PBLs in several cancer patients [46], in line with pre-clinical primate studies [44••,45] and *in vitro* evidence for T helper dependent Vγ9Vδ2 cell proliferation [47]. Interestingly, tumor stabilization or even partial regression was seen in several multiple myeloma patients responding to the Vγ9Vδ2 stimulation protocol [46]. However, unlike healthy non-human primates, about half of treated patients failed to expand their peripheral Vγ9Vδ2 PBLs after treatment with Vγ9Vδ2 agonists and IL-2. Furthermore, repeated treatments led to rapid exhaustion of peripheral proliferative responses of Vγ9Vδ2 T cells in both healthy primates and diseased patients, although the ability of these lymphocytes to respond to Ag *in vitro* seemed to be maintained ([44••] and H Sicard *et al.*, personal communication). Although promising, these observations indicate that immunotherapeutic protocols that target Vγ9Vδ2 lymphocytes need to be optimized before one can conclude about their anti-tumor efficacy. *In vitro* studies suggest that, like IL-2, IL-15 is an important growth and homeostatic factor for Vγ9Vδ2 T cells [48]. Therefore, this cytokine, which can also enhance NKG2D-mediated killing of target cells by both αβ and γδ T cells [49], could be logically tested in combination with Vγ9Vδ2 agonists for immunotherapeutic purposes.

Conclusions

Like other 'non-conventional' T-cell subsets bearing invariant αβ or γδ TCRs, such as CD1d-restricted iNKT cells or murine intraepithelial γδ T-cell subsets, Vγ9Vδ2 T cells exhibit several characteristics that place them at the border between innate and adaptive immunity [50]. Like innate effectors, these lymphocytes recognize conserved Ag upregulated in stressed, infected or transformed cells, acquire early in life a pre-activated status that allows their rapid activation upon Ag encounter, and are found at high frequencies in particular tissue locations in 'pre-immune' individuals. Their implications in diverse physiopathological processes make them particularly interesting targets for immunotherapeutic protocols that aim to either boost or dampen immune responses. Future challenges will be to identify the factors that contribute to the *in vivo* functional polarization and homeostasis of Vγ9Vδ2 T cells in order to set up optimized stimulation protocols allowing long-term induction

of Vγ9Vδ2 T-cell responses with *ad hoc* effector properties. The peculiar reactivity of Vγ9Vδ2 T cells towards antigens upregulated in a wide array of immune and non-immune processes also raises questions about their primary *raison d'être* and their functional complementarity with conventional αβ T cells — issues which should be addressed in future studies.

Acknowledgements

We thank Sophie Maillet, Marie-Claire Devilder and Christophe Gonindard for their expert work in this field and for providing unpublished data (Figure 2). We also thank Innate Pharma (Marseille, France) for kindly providing the phosphoantigens, and Brigitte Gicquel and Nathalie Winter (Institut Pasteur, Paris) for kindly providing the mycobacteria strains. The authors are supported in their research by grants from Institut National de la Sante et de la Recherche Medicale (INSERM), from the Commission of the European Union Programme 6FP (LSH-CT-2003-503367) TB-VAC, from Agence Nationale de la Recherche (ANR; projects #A05118GS and PRIB/017) and from Association pour la Recherche sur le Cancer (ARC; #3662).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hayday AC: **γδ cells: a right time and a right place for a conserved third way of protection**. *Annu Rev Immunol* 2000, **18**:975-1026.

2. Haas W, Pereira P, Tonegawa S: **γ/δ cells**. *Annu Rev Immunol* 1993, **11**:637-685.

3. Chien YH, Jores R, Crowley MP: **Recognition by γ/δ T cells**. *Annu Rev Immunol* 1996, **14**:511-532.

4. Bonneville M, Fournie JJ: **Sensing cell stress and transformation through Vγ9Vδ2 T cell-mediated recognition of the isoprenoid pathway metabolites**. *Microbes Infect* 2005, **7**:503-509.

5. Born WK, Reardon CL, O'Brien RL: **The function of γδ T cells in innate immunity**. *Curr Opin Immunol* 2006, **18**:31-38.

6. De Rosa SC, Andrus JP, Perfetto SP, Mantovani JJ, Herzenberg LA, Roederer M: **Ontogeny of γδ T cells in humans**. *J Immunol* 2004, **172**:1637-1645.

7. Davodeau F, Peyrat MA, Hallet MM, Houde I, Vie H, Bonneville M: **Peripheral selection of antigen receptor junctional features in a major human γδ subset**. *Eur J Immunol* 1993, **23**:804-808.

8. Jomaa H, Feurle J, Luhs K, Kunzmann V, Tony HP, Herderich M, Wilhelm M: **Vγ9/Vδ2 T cell activation induced by bacterial low molecular mass compounds depends on the 1-deoxy-D-xylulose 5-phosphate pathway of isoprenoid biosynthesis**. *FEMS Immunol Med Microbiol* 1999, **25**:371-378.

9. Begley M, Gahan CG, Kollas AK, Hintz M, Hill C, Jomaa H, Eberl M: **The interplay between classical and alternative isoprenoid biosynthesis controls γδ T cell bioactivity of *Listeria monocytogenes***. *FEBS Lett* 2004, **561**:99-104.

10. Tanaka Y, Morita CT, Nieves E, Brenner MB, Bloom BR: **Natural and synthetic non-peptide antigens recognized by human γδ T cells**. *Nature* 1995, **375**:155-158.

11. • Devilder MC, Maillet S, Bouyge-Moreau I, Donnadieu E, Bonneville M, Scotet E: **Potentiation of antigen-stimulated Vγ9Vδ2 T cell cytokine production by immature dendritic cells (DC) and reciprocal effect on DC maturation**. *J Immunol* 2006, **176**:1386-1393.
This study shows that immature DCs are much more potent stimulators of Vγ9Vδ2 T-cell cytokine responses than other APCs, including mature DCs, thus pointing to an important role played by γδ T cells in DC maturation and priming of T-cell responses.

12. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G: **Human T cell receptor γδ cells recognize endogenous mevalonate metabolites in tumor cells**. *J Exp Med* 2003, **197**:163-168.

13. Das H, Groh V, Kuijl C, Sugita M, Morita CT, Spies T, Bukowski JF: **MICA engagement by human Vγ2Vδ2 T cells enhances their antigen-dependent effector function**. *Immunity* 2001, **15**:83-93.

14. Rincon-Orozco B, Kunzmann V, Wrobel P, Kabelitz D, Steinle A, Herrmann T: **Activation of Vγ9Vδ2 T cells by NKG2D**. *J Immunol* 2005, **175**:2144-2151.

15. Viey E, Fromont G, Escudier B, Morel Y, Da Rocha S, Chouaib S, Caignard A: **Phosphostim-activated γδ T cells kill autologous metastatic renal cell carcinoma**. *J Immunol* 2005, **174**:1338-1347.

16. Corvaisier M, Moreau-Aubry A, Diez E, Bennouna J, Mosnier JF, Scotet E, Bonneville M, Jotereau F: **Vγ9Vδ2 T cell response to colon carcinoma cells**. *J Immunol* 2005, **175**:5481-5488.

17. Bukowski JF, Morita CT, Brenner MB: **Human γδ T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity**. *Immunity* 1999, **11**:57-65.

18. • Thompson K, Rojas-Navea J, Rogers MJ: **Alkylamines cause Vγ9Vδ2 T-cell activation and proliferation by inhibiting the mevalonate pathway**. *Blood* 2006, **107**:651-654.
This study demonstrates that, like bisphosphonate, alkylamines activate Vγ9Vδ2 T cells through blockade of the mevalonate pathway and subsequent intracellular IPP accumulation. This provides a unifying view of Vγ9Vδ2 T-cell activation by phosphoantigens.

19. • Poccia F, Agrati C, Martini F, Capobianchi MR, Wallace M, Malkovsky M: **Antiviral reactivities of γδ T cells**. *Microbes Infect* 2005, **7**:518-528.
In this recent review, the authors extensively analyzed the role of γδ T cells in antiviral immunosurveillance.

20. Lang F, Peyrat MA, Constant P, Davodeau F, David-Ameline J, Poquet Y, Vie H, Fournie JJ, Bonneville M: **Early activation of human Vγ9Vδ2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands**. *J Immunol* 1995, **154**:5986-5994.

21. Wadhwa R, Yaguchi T, Hasan MK, Taira K, Kaul SC: **Mortalin–MPD (mevalonate pyrophosphate decarboxylase) interactions and their role in control of cellular proliferation**. *Biochem Biophys Res Commun* 2003, **302**:735-742.

22. •• Scotet E, Martinez LO, Grant E, Barbaras R, Jeno P, Guiraud M, Monsarrat B, Saulquin X, Maillet S, Esteve JP *et al.*: **Tumor recognition following Vγ9Vδ2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I**. *Immunity* 2005, **22**:71-80.
In this study, the authors provide the first evidence for cognate interactions between the Vγ9Vδ2 TCR and AS complex. It describes a new class of tumor antigens derived from mitochondrial enzymes that are recognized by T cells.

23. Burrell HE, Wlodarski B, Foster BJ, Buckley KA, Sharpe GR, Quayle JM, Simpson AW, Gallagher JA: **Human keratinocytes release ATP and utilize three mechanisms for nucleotide interconversion at the cell surface**. *J Biol Chem* 2005, **280**:29667-29676.

24. Ragno S, Romano M, Howell S, Pappin DJ, Jenner PJ, Colston MJ: **Changes in gene expression in macrophages infected with *Mycobacterium tuberculosis*: a combined transcriptomic and proteomic approach**. *Immunology* 2001, **104**:99-108.

25. Moser TL, Kenan DJ, Ashley TA, Roy JA, Goodman MD, Misra UK, Cheek DJ, Pizzo SV: **Endothelial cell surface F1-F0 ATP synthase is active in ATP synthesis and is inhibited by angiostatin**. *Proc Natl Acad Sci USA* 2001, **98**:6656-6661.

26. Monkkonen H, Auriola S, Lehenkari P, Kellinsalmi M, Hassinen IE, Vepsalainen J, Monkkonen J: **A new endogenous ATP analog (Apppl) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates**. *Br J Pharmacol* 2006, **147**:437-445.

27. Constant P, Davodeau F, Peyrat MA, Poquet Y, Puzo G, Bonneville M, Fournie JJ: **Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands**. *Science* 1994, **264**:267-270.

28. Glatzel A, Wesch D, Schiemann F, Brandt E, Janssen O, Kabelitz D: **Patterns of chemokine receptor expression on peripheral blood γδ T lymphocytes: strong expression of CCR5 is a selective feature of Vδ2/Vγ9 γδ T cells**. *J Immunol* 2002, **168**:4920-4929.

29. Brandes M, Willimann K, Lang AB, Nam KH, Jin C, Brenner MB, Morita CT, Moser B: **Flexible migration program regulates γδ T-cell involvement in humoral immunity**. *Blood* 2003, **102**:3693-3701.

30. Battistini L, Caccamo N, Borsellino G, Meraviglia S, Angelini DF, Dieli F, Cencioni MT, Salerno A: **Homing and memory patterns of human γδ T cells in physiopathological situations**. *Microbes Infect* 2005, **7**:510-517.

31. Brigl M, Brenner MB: **CD1: antigen presentation and T cell function**. *Annu Rev Immunol* 2004, **22**:817-890.

32. Munz C, Steinman RM, Fujii S: **Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity**. *J Exp Med* 2005, **202**:203-207.

33. Conti L, Casetti R, Cardone M, Varano B, Martino A, Belardelli F, Poccia F, Gessani S: **Reciprocal activating interaction between dendritic cells and pamidronate-stimulated γδ T cells: role of CD86 and inflammatory cytokines**. *J Immunol* 2005, **174**:252-260.

34. • Collins C, Wolfe J, Roessner K, Shi C, Sigal LH, Budd RC: **Lyme arthritis synovial γδ T cells instruct dendritic cells via fas ligand**. *J Immunol* 2005, **175**:5656-5665.
This study provides a demonstration of an unexpected mechanism of DC maturation, which is mediated by γδ T cells through Fas engagement.

35. Yamashita S, Tanaka Y, Tsutsumi S, Aburatani H, Minato N, Ihara S: **Analysis of mechanism for human γδ T cell recognition of nonpeptide antigens**. *Biochem Biophys Res Commun* 2005, **334**:349-360.

36. • Brandes M, Willimann K, Moser B: **Professional antigen-presentation function by human γδ T cells**. *Science* 2005, **309**:264-268.
The authors report that activated Vγ9Vδ2 T cells, like classical and well described APCs, such as DCs, can not only upregulate surface co-stimulators but also efficiently process and present antigens. These unexpected results thus suggest that Vγ9Vδ2 T cells could directly participate in the induction of adaptative immune response, by priming of naïve αβ T cells.

37. Wesch D, Glatzel A, Kabelitz D: **Differentiation of resting human peripheral blood γδ T cells toward Th1- or Th2-phenotype**. *Cell Immunol* 2001, **212**:110-117.

38. Ordway DJ, Pinto L, Costa L, Martins M, Leandro C, Viveiros M, Amaral L, Arroz MJ, Ventura FA, Dockrell HM: **γδ T cell responses associated with the development of tuberculosis in health care workers**. *FEMS Immunol Med Microbiol* 2005, **43**:339-350.

39. Workalemahu G, Foerster M, Kroegel C: **Expression and synthesis of fibroblast growth factor-9 in human γδ T-lymphocytes. Response to isopentenyl pyrophosphate and TGF-β1/IL-15**. *J Leukoc Biol* 2004, **75**:657-663.

40. Workalemahu G, Foerster M, Kroegel C: **Expression of metalloproteinase-7 (matrilysin) in human blood and bronchoalveolar γδ T-lymphocytes. Selective upregulation by the soluble non-peptidic mycobacterial phosphoantigen (isopentenyl pyrophosphate)**. *J Cell Physiol* 2006, **207**:67-74.

41. Jameson JM, Sharp LL, Witherden DA, Havran WL: **Regulation of skin cell homeostasis by γδ T cells**. *Front Biosci* 2004, **9**:2640-2651.

42. Kabelitz D, Wesch D, Pitters E, Zoller M: **Characterization of tumor reactivity of human Vγ9Vδ2 γδ T cells *in vitro* and in SCID mice *in vivo***. *J Immunol* 2004, **173**:6767-6776.

43. Lozupone F, Pende D, Burgio VL, Castelli C, Spada M, Venditti M, Luciani F, Lugini L, Federici C, Ramoni C *et al.*: **Effect of human natural killer and γδ T cells on the growth of human autologous melanoma xenografts in SCID mice**. *Cancer Res* 2004, **64**:378-385.

44. •• Sicard H, Ingoure S, Luciani B, Serraz C, Fournie JJ, Bonneville M, Tiollier J, Romagne F: ***In vivo* immunomanipulation of Vγ9Vδ2 T cells with a synthetic phosphoantigen in a preclinical nonhuman primate model**. *J Immunol* 2005, **175**:5471-5480.
This article demonstrates efficient *in vivo* expansion and activation of Vγ9Vδ2 T cells following injection of a synthetic phosphoAg and IL-2 in monkeys, as well as exhaustion of Vγ9Vδ2 T cell responses upon repeated treatments. This forms the basis of subsequent clinical trials that aim to use phosphoAg to activate Vγ9Vδ2 T cells.

45. Casetti R, Perretta G, Taglioni A, Mattei M, Colizzi V, Dieli F, D'Offizi G, Malkovsky M, Poccia F: **Drug-induced expansion and differentiation of Vγ9Vδ2 T cells *in vivo*: the role of exogenous IL-2**. *J Immunol* 2005, **175**:1593-1598.

46. Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, Tony HP: **γδ T cells for immune therapy of patients with lymphoid malignancies**. *Blood* 2003, **102**:200-206.

47. Wesch D, Kabelitz D, Friese K, Pechhold K: **Mycobacteria-reactive γδ T cells in HIV-infected individuals: lack of Vγ9 cell responsiveness is due to deficiency of antigen-specific CD4 T helper type 1 cells**. *Eur J Immunol* 1996, **26**:557-562.

48. Caccamo N, Meraviglia S, Ferlazzo V, Angelini D, Borsellino G, Poccia F, Battistini L, Dieli F, Salerno A: **Differential requirements for antigen or homeostatic cytokines for proliferation and differentiation of human Vγ9Vδ2 naïve, memory and effector T cell subsets**. *Eur J Immunol* 2005, **35**:1764-1772.

49. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T *et al.*: **Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease**. *Immunity* 2004, **21**:357-366.

50. Bendelac A, Bonneville M, Kearney JF: **Autoreactivity by design: innate B and T lymphocytes**. *Nat Rev Immunol* 2001, **1**:177-186.