

Antigen Recognition by Human $\gamma\delta$ T Lymphocytes

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Key Words

$\gamma\delta$ T cells · T cell receptor repertoire · Phosphoantigens · MHC restriction · MHC class I-related chain

Abstract

Mature T lymphocytes carrying the conventional $\alpha\beta$ T cell receptor (TCR) recognize peptide antigens in the context of MHC class I (CD8+) and MHC class II (CD4+) antigens, respectively. In striking contrast, human $\gamma\delta$ T lymphocytes recognize unconventional antigens via their heterodimeric TCR in a non-MHC-restricted fashion. In this brief review, we discuss recent progress in the identification of ligands that are specifically recognized by human $\gamma\delta$ T cells. It appears that $\gamma\delta$ T cells have evolved to supplement the cellular immune response towards antigens that are not seen by $\alpha\beta$ T lymphocytes.

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function of these $\gamma\delta$ T cells under physiological and pathological conditions has grown continuously since the discovery more than 15 years ago that a third rearranging (γ chain) TCR gene is expressed in a distinct set of T lymphocytes [1]. Despite tremendous investigative efforts, however, the function of $\gamma\delta$ T cells has remained largely elusive. This is due to the fact that it has been difficult to assign to $\gamma\delta$ T cells a specific role in the immune system. More recently, however, new ligands for $\gamma\delta$ T cells have been identified. Recent progress in the field supports the idea that $\gamma\delta$ T cells fulfill a role distinct from that of $\alpha\beta$ T cells in the immune system. In this brief review, we attempt to discuss some of these new developments. It is not the aim of this article to provide an in-depth review on $\gamma\delta$ T cells. For this purpose, the reader is referred to recent reviews dealing with the $\gamma\delta$ TCR repertoire in health and disease [2, 3] and functional aspects of $\gamma\delta$ T cells [4, 5].

Introduction

While the vast majority of mature T lymphocytes carry a T cell receptor (TCR) molecule composed of α and β chains, a small percentage (1–5%) expresses an alternative CD3-associated $\gamma\delta$ TCR heterodimer. Interest in the

The Human $\gamma\delta$ TCR Repertoire

In contrast to $\alpha\beta$ T cells, only a few variable (V) gene segments are available in the germline genome from which functional γ and δ chain genes can be generated. Thus, there are 6 expressed V γ genes (designated V γ 2, 3, 4, 5, 8 and 9) and a comparably small number of V δ genes.

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Nevertheless, the $\gamma\delta$ TCR repertoire is considered to be at least as diverse as the $\alpha\beta$ TCR repertoire, due to nongerm-line-encoded mechanisms of diversification such as the insertion of N nucleotides during gene rearrangement and the translation of D δ sequences in all three possible reading frames [3]. Over the last few years, monoclonal antibodies directed against expressed TCR V γ and V δ genes have been developed. Such antibodies have proven useful in the analysis of the human $\gamma\delta$ TCR repertoire in health and disease [2, 6, 7]. Quite strikingly, the few available V γ and V δ genes are not used randomly among the $\gamma\delta$ T cells in different anatomical localizations. In the peripheral blood of healthy Caucasians, there is a clear dominance of cells expressing V γ 9 together with V δ 2, which can account for up to 95% of the $\gamma\delta$ T cells [3, 6, 8]. While V γ 9/V δ 2 cells do not dominate among $\gamma\delta$ T cells after birth, they gradually expand in the peripheral blood during childhood, suggesting that exposure to environmental and/or bacterial antigens (see below) might shape the $\gamma\delta$ TCR repertoire during postnatal development [3, 8]. The second most frequent subset of peripheral blood $\gamma\delta$ T cells expresses V δ 1 in association with various V γ elements. However, due to the dominance of V γ 9/V δ 2 cells, the V δ 1 subset usually accounts for only 10–30 % of the peripheral blood $\gamma\delta$ T cells, and might be difficult to detect given the low percentage of $\gamma\delta$ T cells among CD3⁺ peripheral blood T cells. Quantitative increases in circulating V δ 1 cells are observed in HIV infection [2, 7, 9] and, even more strikingly, in cytomegalovirus (CMV) infection following renal transplantation [10]. Under physiological conditions, however, the primary localization of V δ 1-expressing $\gamma\delta$ T cells is not the peripheral blood but rather the intraepithelial T cell compartment of the intestine, where V δ 1 cells constitute a major T cell population [11]. The preferential expression of certain TCR V γ and V δ elements in different anatomical sites is usually considered to reflect the importance of a particular $\gamma\delta$ T cell subset in the local immune surveillance.

Although the overall structure of $\gamma\delta$ and $\alpha\beta$ TCRs is similar, the $\gamma\delta$ TCR displays some distinct structural features. Recent crystal structure analysis of the V δ domain of a human $\gamma\delta$ TCR revealed that the framework structure of V δ more closely resembled that of the variable immunoglobulin heavy chain domain (V_H) than the TCR V α or V β domains [12]. Together with the observation that the CDR3 length distribution of TCR δ chains is similar to that of immunoglobulin heavy chains [13], it appears that ligand recognition by $\gamma\delta$ T cells shares more features with antigen binding of immunoglobulins than peptide/MHC recognition by $\alpha\beta$ T cells [14, 15].

Ligands Recognized by Human V γ 9/V δ 2 T Cells

Mature $\alpha\beta$ T cells recognize antigens processed by antigen-presenting cells in the context of MHC class I or class II molecules. While CD4⁺ cells recognize peptides derived from exogenous antigens bound to MHC class II molecules, CD8⁺ T cells recognize peptides in the context of MHC class I molecules. In contrast to mature $\alpha\beta$ T cells, the majority of $\gamma\delta$ T cells lack the expression of CD4 or CD8, which is in agreement with the lack of MHC restriction of most $\gamma\delta$ T cells. Thus, only a few examples of MHC-restricted human $\gamma\delta$ T cell clones have been reported [16]. Early in vitro experiments indicated that the dominant subset of peripheral blood $\gamma\delta$ T cells, i.e. V γ 9/V δ 2-expressing cells, strongly reacted in a non-MHC-restricted fashion towards inactivated *Mycobacterium tuberculosis* [17, 18] and a variety of other microorganisms, including *Plasmodium falciparum* [19], *Toxoplasma gondii* [20], *Yersinia enterocolitica* [21], *Francisella tularensis* [22] and others. Further investigations revealed that the $\gamma\delta$ T cell-stimulating moiety of microbial extracts was not protein, but rather consisted of nonproteinaceous, phosphatase-sensitive, low-molecular-weight compounds [23]. Different types of such phosphorylated ligands have been isolated from mycobacteria, including phosphorylated nucleotide-containing compounds (TUBag) [24] and isoprenoid pyrophosphates such as isopentenyl pyrophosphate (IPP) [25]. Collectively, these ligands have been termed phosphoantigens [26]. Apart from these TUBags and pyrophosphates, several phosphorylated sugars and synthetic alkyl phosphates such as monoethyl phosphate have been found to stimulate human V γ 9/V δ 2 T cells [27, 28]. It is now obvious that phosphoantigens can be produced by a large variety of microorganisms [22, 24, 25, 29], and $\gamma\delta$ T cell-stimulating, phosphatase-sensitive, protease-resistant low-molecular-weight compounds can also be recovered from certain plant extracts [30]. Since IPP is a precursor of a biosynthesis pathway leading to the synthesis of diverse isoprenoids, including vitamins and steroids, both in prokaryotic and eukaryotic cells [31], it has been unclear why V γ 9/V δ 2 T cells react towards synthetic IPP and IPP derived from microorganisms but not towards extracts or lysates of eukaryotic and autologous cells where IPP is also synthesized. Recent studies from two groups have provided new insights into this puzzling problem. Importantly, IPP can be synthesized via two different biosynthetic pathways. In eukaryotic cells, including mammalian cells and yeast, as well as in some bacteria, IPP is synthesized from acetyl coenzyme A via the formation of mevalonic acid as an intermediate step (me-

valonate pathway) [31]. In other bacteria (including *M. tuberculosis*), however, IPP synthesis does not start from acetyl coenzyme A but rather arises from the decarboxylate condensation of glyceraldehyde-3-phosphate and pyruvate, leading to 1-deoxy-*D*-xylulose 5-phosphate (DXP) and via additional intermediates to IPP (non-mevalonate pathway) [32–34]. Jomaa et al. [35] have compared the $\gamma\delta$ T cell-stimulatory activity of a large number of different bacteria with their capacity to use the mevalonate or non-mevalonate (DXP) pathway of IPP biosynthesis. Interestingly, they observed a close correlation, in that $\gamma\delta$ T cell-stimulatory activity was strictly confined to bacteria exploiting the DXP but not the mevalonate pathway [35]. Moreover, Belmont et al. [36] were able to identify a novel intermediate of this nonmevalonate pathway as the major mycobacteria-derived $\gamma\delta$ T cell-stimulating metabolite. In their recent studies, they showed that a pyrophosphorylated aldehyde, 3-formyl-1-butyl-pyrophosphate, is generated exclusively during the nonmevalonate (DXP) pathway of IPP synthesis, and that it activates V γ 9/V δ 2 T cells at the nanomolar range, while IPP itself is active only at much higher concentrations [36]. These new results help us to understand the role of V γ 9/V δ 2 T cells in the immune defense against pathogenic microorganisms. Accordingly, V γ 9/V δ 2 T cells recognize metabolites of isoprenoid synthesis that are generated by the nonmevalonate pathway in certain pathogenic microorganisms but not by the mevalonate pathway in other bacteria and mammalian cells. IPP as the end product of both biosynthesis pathways, activates V γ 9/V δ 2 T cells only at 1,000-fold higher concentrations (i.e. in the micromolar range), which, however, are not achieved in mammalian cells. Therefore, V γ 9/V δ 2 T cells sense proliferating pathogens that secrete very low amounts of intermediates of the non-mevalonate isoprenoid biosynthesis pathway [36].

In addition to phosphoantigens, primary alkylamines have recently been demonstrated to activate the same subset of V γ 9/V δ 2 $\gamma\delta$ T cells. Such alkylamines are secreted by some bacteria and are also found in certain plants and tea [37]. Furthermore, V γ 9/V δ 2 T cells also recognize certain tumor cells, such as Daudi Burkitt's lymphoma cells [38]. While it has been shown that a GroEL-related heat shock protein (HSP) might be recognized by $\gamma\delta$ T cells on the surface of Daudi cells [39], more recent data suggest that the surface-expressed V γ 9/V δ 2 ligand is still elusive [40]. As with other T cells and NK cells, the activation pattern of $\gamma\delta$ T cells can be modulated by signals delivered through MHC class I-specific inhibitory receptors. A substantial fraction of V γ 9/V δ 2 T cells expresses CD94/NKG2A receptors, which can modulate

reactivity towards lymphoma cells and phosphoantigens [41, 42].

The effector function of V γ 9/V δ 2 T cells includes cytokine secretion (tumor necrosis factor- α , interferon- γ , CC chemokines and others) and cytotoxic activity against pathogen-infected macrophages [4, 43–45]. Gene transfer experiments have clearly shown that the recognition of phosphoantigens by V γ 9/V δ 2 T cells is TCR dependent [46]. However, in contrast to peptides recognized by $\alpha\beta$ T cells, the presentation of phosphoantigens to human V γ 9/V δ 2 T cells occurs independently of classical MHC class I or class II antigens, or MHC-related CD1 molecules, and does not require functional peptide-loading pathways [47]. This lack of requirement for processing ensures that V γ 9/V δ 2 $\gamma\delta$ T cells can rapidly respond to phosphoantigens secreted by pathogenic microorganisms.

Ligands Recognized by Human V δ 1 T Cells

$\gamma\delta$ T cells expressing V δ 1 in association with various V γ elements are preferentially located in the intestine. In the adult intestine, $\gamma\delta$ T cells comprise 30–40% of intraepithelial lymphocytes, of which up to 70% are V δ 1+ [3, 48]. The accumulation of V δ 1 cells in the intestine suggests that these cells fulfill important functions in the local mucosal immune surveillance. This is further supported by the observation that quantitative and qualitative changes in the mucosal $\gamma\delta$ T cell repertoire and function occur in inflammatory bowel diseases [49–52]. Recently, candidate target antigens for mucosal V δ 1-expressing $\gamma\delta$ T cells have been identified. As reported by two groups, V δ 1 $\gamma\delta$ T cells coexpressing various V γ genes recognized the stress-inducible MHC class I-related chain (MIC) A and MICB molecules on the surface of epithelial cells [53, 54]. The MIC genes encode cell surface glycoproteins which are not associated with β_2 -microglobulin, lack a conventional peptide-binding groove (and thus are stable in the absence of MHC class I peptide ligand) and are preferentially expressed in gastrointestinal epithelium upon stress, e.g. infection, cell damage or cellular transformation [55, 56]. These data suggest that a major role of intraepithelial V δ 1 $\gamma\delta$ T cells is to monitor the integrity of epithelial cells by sensing the expression of MICA/MICB in the case of cell damage. Interestingly enough, MICA/MICB antigens are aberrantly expressed also on certain epithelial tumor cells, and the expression of MICA/MICB on a given tumor correlates with the appearance of V δ 1 $\gamma\delta$ T cells among tumor-infiltrating lymphocytes [57]. These data suggest that V δ 1 $\gamma\delta$ T cells might be involved in the

cellular immune response towards MICA/MICB-expressing epithelial tumor cells.

While the stress-inducible expression of MICA/MICB molecules on epithelial cells suggests a role of intraepithelial V δ 1-expressing $\gamma\delta$ T cells in the local immune surveillance, there is evidence that these cells are also involved in the immune defense against infection. V δ 1 T cells are increased in the peripheral blood of HIV-infected individuals, but the significance of this observation is uncertain, and potential ligands involved have not yet been identified [2]. More recently, V δ 1 T cells have been implicated in the human immune response to CMV. Dechanet et al. [10] studied the $\gamma\delta$ T cells in the peripheral blood of patients undergoing renal transplantation. After transplantation, they observed a striking increase in V δ 1 cells in those patients who developed a CMV infection but not in patients who remained free of CMV infection [10]. This increase in V δ 1 cells was long lasting (more than 1 year) and was characterized by the clonal expansion of particular V δ 1 (and V δ 3) clones, suggesting an antigen-driven process. These data strongly suggest that the subset of V δ 1 $\gamma\delta$ T cells plays an important role in the immune response of immunosuppressed patients to CMV infection.

Additional evidence indicates that V δ 1-expressing $\gamma\delta$ T cells are also involved in the immune defense against certain bacteria. $\gamma\delta$ T cells present in the synovial fluid of patients with Lyme arthritis proliferate in vitro in response to *Borrelia burgdorferi*, the causative spirochete [58]. In their studies, Vincent et al. [58] demonstrated that *B. burgdorferi*-reactive V δ 1 T cells can eliminate autologous synovial CD4⁺ T lymphocytes via a Fas/Fas-ligand interaction. Such a mechanism could help to restrict the expansion of inflammatory CD4⁺ T cells in the inflamed synovium [58]. Similarly, we observed a profound expansion of V δ 1 $\gamma\delta$ T cells coexpressing diverse V γ elements when synovial lymphocytes from Lyme arthritis patients were cultured with extracts of *B. burgdorferi*. In an attempt to investigate the possible role of V δ 1 $\gamma\delta$ T cells in other manifestations of *Borrelia* infection, we quantified $\gamma\delta$ T cell subsets in the peripheral blood of patients with skin manifestations of a previously documented *Borrelia* infection. Flow cytometric analysis of peripheral blood lymphocytes from these patients did not reveal increased percentages of V δ 1 cells when compared to healthy individuals without documented *Borrelia* infection. Functional studies indicated that the strong proliferative response of lymphocytes from these patients to extracts of various *Borrelia* species (*B. garinii*, *B. afzelii*, *B. burgdorferi*) was primarily due to the activation of $\alpha\beta$ T lympho-

cytes. Thus, a selective expansion of V δ 1 $\gamma\delta$ T cells as described with synovial lymphocytes from Lyme arthritis patients could not be observed with peripheral blood lymphocytes from these patients with skin manifestations of *Borrelia* infection [Glatzel et al., unpubl. results]. It is conceivable that the reactivity of V δ 1 $\gamma\delta$ T cells towards *Borrelia* antigens is a feature of tissue-localized (e.g. synovial) but not of circulating V δ 1 $\gamma\delta$ T cells. More recent data from Vincent et al. [59] indicate that the V δ 1-expressing $\gamma\delta$ T cells isolated from the synovial fluid of Lyme arthritis patients might recognize lipoproteins and lipidated hexapeptides derived from *B. burgdorferi* outer surface proteins.

Taken together, the two major subsets of human $\gamma\delta$ T cells seem to recognize fundamentally different ligands. While the prominent set of V γ 9/V δ 2 T cells recognizes nonproteinaceous phosphoantigens and alkylamines, the V δ 1 $\gamma\delta$ T cells might recognize more conventional antigens, including proteins (MICA/MICB), lipoproteins/lipidated peptides (*B. burgdorferi*) and not yet precisely identified viral antigens (CMV). Interestingly enough, responsiveness to phosphoantigens seems to be limited to human $\gamma\delta$ T cells, whereas murine $\gamma\delta$ T cells fail to recognize these ligands [5]. While murine $\gamma\delta$ T cells recognize mycobacterial HSP and HSP-derived peptides [60], the role of HSPs as ligands for human $\gamma\delta$ T cells is less clear. Although there are examples of HSP-reactive human $\gamma\delta$ T cells [39, 61, 62], it would appear that HSPs are not universal ligands for human $\gamma\delta$ T cells [18]. Phosphoantigens and MICA/MICB share properties of superantigens, i.e. they stimulate whole subsets of $\gamma\delta$ T cells as defined by their V gene usage (V γ 9/V δ 2 in the case of phosphoantigens, V δ 1 in the case of MICA/MICB). In addition to this supertypic reactivity, individual $\gamma\delta$ T cell clones might display additional specificities (e.g. for HSP) which would depend on the hypervariable CDR3 loop and adjacent residues [63].

Possible Functions of $\gamma\delta$ T Cells

The above-summarized new insights into ligands recognized by human $\gamma\delta$ T cells strongly indicate that a major role of $\gamma\delta$ T cells is to supplement the cellular immune defense towards antigens that are not seen by $\alpha\beta$ T cells. This is true for V γ 9/V δ 2 $\gamma\delta$ T cells with their unique capacity to sense very low amounts of metabolites that are secreted by pathogenic microorganisms such as *M. tuberculosis*, as well as for mucosal V δ 1 $\gamma\delta$ T cells that recognize stress-induced MICA/MICB antigens. Accumulating evi-

dence suggests, however, that $\gamma\delta$ T cells possess additional immunoregulatory functions [see ref. 5 for a recent review]. Cross-talk between $\gamma\delta$ T cells and other cells of the immune system has been shown to modulate humoral [64, 65] and cellular immune responses [66–69]. Our own in vitro experiments indicate a bidirectional interaction between phosphoantigen-reactive V γ 9/V δ 2 $\gamma\delta$ T cells and CD4+ $\alpha\beta$ T cells. While the activation of freshly isolated V γ 9/V δ 2 T cells by heat-inactivated *M. tuberculosis* or synthetic IPP phosphoantigens totally depends on CD4+-derived helper signals such as IL-2 [70, 71], the simultaneous activation of CD4+ $\alpha\beta$ T cells by bacterial superantigens can paradoxically suppress V γ 9/V δ 2 $\gamma\delta$ T cell activation by an as yet undefined mode of action [Wesch et al., unpubl. observations]. In addition, $\gamma\delta$ T cells might exert more specific immunoregulatory functions that cannot be readily achieved by $\alpha\beta$ T cells. An example is the role of $\gamma\delta$ T cells in allergic airway inflammation. In a murine model of allergic pulmonary inflammation, Zuan-ny-Amorim et al. [72] showed that $\gamma\delta$ T cells are essential

for inducing IL-4-dependent IgE and IgG1 responses and for Th2-mediated airway inflammation to peptide antigens. It appears, however, that the role of $\gamma\delta$ T cells in the regulation of airway function is not restricted to immune-dependent mechanisms but might also include interactions with nonimmune cells such as airway epithelia and airway smooth muscle cells [73].

Taken together, these results make it increasingly clear why the immune system has been able to afford to maintain two separate TCRs throughout evolution. While the identification of specific $\gamma\delta$ T cell ligands has progressed rapidly, it is a challenge for the future to define the basis for the immunoregulatory functions of $\gamma\delta$ T cells at the molecular level.

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References

- Saito H, Kranz DM, Tagagaki Y, Hayday AC, Eisen HN, Tonegawa S: Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature* 1984; 309:757–762.
- Kabelitz D, Wesch D, Hinz T: $\gamma\delta$ T cells, their T cell receptor usage and role in human diseases. *Springer Semin Immunopathol* 1999;21: 55–75.
- McVay LD, Carding SR: Generation of human $\gamma\delta$ T-cell repertoires. *Crit Rev Immunol* 1999; 19:431–460.
- Kabelitz D: Effector functions and control of human $\gamma\delta$ T-cell activation. *Microbes Infect* 1999;1:255–261.
- Born W, Cady C, Jones-Carson J, Mukasa A, Lahn M, O'Brien R: Immunoregulatory functions of $\gamma\delta$ T cells. *Adv Immunol* 1999;71:77–144.
- Hinz T, Wesch D, Halary F, Marx S, Choudhary A, Arden B, Janssen O, Bonneville M, Kabelitz D: Identification of the complete expressed human TCR V γ repertoire by flow cytometry. *Int Immunol* 1997;9:1065–1072.
- Wesch D, Hinz T, Kabelitz D: Analysis of the TCR V γ repertoire in healthy donors and HIV-1-infected individuals. *Int Immunol* 1998;10: 1067–1075.
- Parker CM, Groh V, Band H, Porcelli SA, Morita G, Fabbri M, Glass D, Strominger JL, Brenner MB: Evidence for extrathymic changes in the T-cell receptor $\gamma\delta$ repertoire. *J Exp Med* 1990;171:1597–1612.
- Boullier S, Cochet M, Poccia F, Gougeon M-L: CDR3-independent $\gamma\delta$ V δ 1+ T cell expansion in the peripheral blood of HIV-infected persons. *J Immunol* 1995;154:1418–1431.
- Dechanet J, Merville P, Lim A, Retiere C, Pitard V, Lafarge X, Michelson S, Meric C, Hallet M-M, Kourilsky P, Potaux L, Bonneville M, Moreau J-F: Implication of $\gamma\delta$ T cells in the human immune response to cytomegalovirus. *J Clin Invest* 1999;103:1437–1449.
- Porcelli S, Brenner MB, Band H: Biology of the human $\gamma\delta$ T-cell receptor. *Immunol Rev* 1991; 120:137–183.
- Li H, Lebedeva MI, Llera AS, Fields BA, Brenner MB, Mariuzza RA: Structure of the V δ domain of a human $\gamma\delta$ T-cell antigen receptor. *Nature* 1998;391:502–506.
- Rock EP, Sibbald PR, Davis MM, Chien YH: CDR3 length in antigen-specific immune responses. *J Exp Med* 1994;179:323–328.
- Schild H, Mavaddat N, Litzenger C, Ehrlich EW, Davis MM, Bluestone JA, Matis L, Draper RK, Chien YH: The nature of major histocompatibility complex recognition by gamma delta T cells. *Cell* 1994;76:29–37.
- Crowley MP, Reich Z, Mavaddat N, Altman JD, Chien Y: The recognition of the nonclassical major histocompatibility complex (MHC) class I molecule, T10, by the gamma delta T cell, G8. *J Exp Med* 1997;185:1223–1230.
- Kozbor D, Trinchieri G, Monos DS, Isobe M, Russo G, Haney JA, Zmijewski C, Croce CM: Human TCR- $\gamma^*\delta^+$, CD8+ T lymphocytes recognize tetanus toxoid in an MHC-restricted fashion. *J Exp Med* 1989;169:1847–1851.
- Pfeffer K, Schoel B, Gulle B, Kaufmann SH, Wagner H: Primary responses of human T cells to mycobacteria: A frequent set of gamma/delta T cells are stimulated by protease-resistant ligands. *Eur J Immunol* 1990;20:1175–1179.
- Kabelitz D, Bender A, Schondelmaier S, Schoel B, Kaufmann SH: A large fraction of human peripheral blood gamma/delta+ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. *J Exp Med* 1990;171:667–679.
- Behr C, Dubois P: Preferential expansion of V gamma 9 V delta 2 T cells following stimulation of peripheral blood lymphocytes with extracts of *Plasmodium falciparum*. *Int Immunol* 1992;4:361–366.
- Subauste CS, Chung JY, Do D, Koniaris AH, Hunter CA, Montoya JG, Porcelli S, Remington JS: Preferential activation and expansion of human peripheral blood $\gamma\delta$ T cells in response to *Toxoplasma gondii* in vitro and their cytokine production and cytotoxic activity against *T. gondii*-infected cells. *J Clin Invest* 1995;96:610–619.
- Young JL, Goodall JC, Beacock-Sharp H, Gaston JS: Human $\gamma\delta$ T-cell recognition of *Yersinia enterocolitica*. *Immunology* 1997;91:503–510.
- Poquet Y, Kroca M, Halary F, Stenmark S, Peyrat MA, Bonneville M, Fournie JJ, Sjostedt A: Expansion of Vgamma9 Vdelta2 T cells is triggered by *Francisella tularensis*-derived phosphoantigens in tularemia but not after tularemia vaccination. *Infect Immun* 1998;66: 2107–2114.

- 23 Schoel B, Sprenger S, Kaufmann SH: Phosphate is essential for stimulation of Vgamma9 Vdelta2 T lymphocytes by mycobacterial low molecular weight ligand. *Eur J Immunol* 1994; 24:1886–1892.
- 24 Constant P, Davodeau F, Peyrat MA, Poquet Y, Puzo G, Bonneville M, Fournie JJ: Stimulation of human gamma delta T cells by nonpeptidic mycobacterial ligands. *Science* 1994;264: 267–270.
- 25 Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR: Natural and synthetic non-peptide antigens recognized by human $\gamma\delta$ T cells. *Nature* 1995;375:155–158.
- 26 Fournie JJ, Bonneville M: Stimulation of gamma delta T cells by phosphoantigens. *Res Immunol* 1996;147:338–346.
- 27 De Libero G: Sentinel function of broadly reactive human gamma delta T cells. *Immunol Today* 1997;18:2272–2279.
- 28 Tanaka Y, Sano S, Nieves E, de Libero G, Rosa D, Modlin RL, Brenner MB, Bloom BR, Morita CT: Nonpeptide ligands for human gamma delta T cells. *Proc Natl Acad Sci USA* 1994;91: 8175–8179.
- 29 Behr C, Poupot R, Peyrat MA, Poquet Y, Constant P, Dubois P, Bonneville M, Fournie JJ: *Plasmodium falciparum* stimuli for human gamma delta T cells are related to phosphorylated antigens of mycobacteria. *Infect Immun* 1996;64:2892–2896.
- 30 Fischer S, Scheffler A, Kabelitz D: Activation of human gamma delta T cells by heat-treated mistletoe extracts. *Immunol Lett* 1996;52:69–72.
- 31 Betyia ED, Porter JW: Biochemistry of polyisoprenoid biosynthesis. *Annu Rev Biochem* 1976;45:113–142.
- 32 Rohmer M, Knani M, Simonin P, Sutter B, Sahm H: Isoprenoid biosynthesis in bacteria: A novel pathway for early steps leading to isopen-tenyl diphosphate. *Biochem J* 1993;295:517–524.
- 33 Horbach S, Sahm H, Welle R: Isoprenoid biosynthesis in bacteria: Two different pathways? *FEMS Microbiol Lett* 1993;111:135–140.
- 34 Rohmer M: The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Nat Prod Rep* 1999;16:565–574.
- 35 Jomaa H, Feurle J, Lühs K, Kunzmann V, Tony H-P, Herderich M, Wilhelm M: V γ 9/V δ 2 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.
- 36 Belmant C, Espinosa E, Poupot R, Peyrat M-A, Guiraud M, Poquet Y, Bonneville M, Fournie JJ: 3-Formyl-1-butyl pyrophosphate: A novel mycobacterial metabolite-activating human $\gamma\delta$ T cells. *J Biol Chem* 1999;274:32079–32084.
- 37 Bukowski JF, Morita CT, Brenner MB: Human gamma delta T cells recognize alkylamines derived from microbes, edible plants, and tea: Implications for innate immunity. *Immunity* 1999;11:57–65.
- 38 Sturm E, Braakman E, Fisch P, Vreugdenhil RJ, Sondel P, Bolhuis RLH: Human V γ 9-V δ 2 T cell receptor- $\gamma\delta$ lymphocytes show specificity to Daudi Burkitt's lymphoma cells. *J Immunol* 1990;145:3202–3208.
- 39 Fisch P, Malkovsky M, Kovats S, Sturm E, Braakman E, Klein BS, Voss SD, Morrissey LW, De Mars R, Welch WJ, Bolhuis RLH, Sondel PM: Recognition by human V γ 9/V δ 2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. *Science* 1990;250:1269–1273.
- 40 L'Faqihi FE, Guiraud M, Dastugue N, Brousset P, Le Bouteiller P, Halary F, Bonneville M, Fournie JJ, Champagne E: Acquisition of a stimulatory activity for Vgamma9/Vdelta2 T cells by a Burkitt's lymphoma cell line without loss of HLA class I expression. *Hum Immunol* 1999;60:928–938.
- 41 Fisch P, Meuer E, Pende D, Rothenfusser S, Viale O, Kock S, Ferrone S, Fradelizi D, Klein G, Moretta L, Rammensee HG, Boon T, Coulie P, van der Bruggen P: Control of B cell lymphoma recognition via natural killer inhibitory receptors implies a role for human V γ 9/V δ 2 T cells in tumor immunity. *Eur J Immunol* 1997; 27:3368–3379.
- 42 Boullier S, Poquet Y, Halary F, Bonneville M, Fournie JJ, Gougeon ML: Phosphoantigen activation induces surface translocation of intracellular CD94/NKG2A class I receptor on CD94-peripheral Vgamma9 Vdelta2 T cells but not on CD94-thymic or mature gammadelta T cell clones. *Eur J Immunol* 1998;28:3399–3410.
- 43 Lang F, Peyrat MA, Constant P, Davodeau F, David-Ameline J, Poquet Y, Vie H, Fournie JJ, Bonneville M: Early activation of human V gamma 9 V delta 2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. *J Immunol* 1995;154:5986–5994.
- 44 Poccia F, Battistini L, Cipriani B, Mancino G, Martini F, Gougeon ML, Colizzi V: Phosphoantigen-reactive Vgamma9 Vdelta2 T lymphocytes suppress in vitro human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. *J Infect Dis* 1999;180:858–861.
- 45 Tsukaguchi K, Balaji KN, Boom WH: CD4+ alpha beta T cell and gamma delta T cell responses to *Mycobacterium tuberculosis*. Similarities and differences in Ag recognition, cytotoxic effector function, and cytokine production. *J Immunol* 1995;154:1786–1796.
- 46 Bukowski JF, Morita CT, Tanaka Y, Bloom BR, Brenner MB, Band H: V gamma 2 V delta 2 TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. *J Immunol* 1995;154:998–1006.
- 47 Morita CT, Beckman EM, Bukowski JF, Tanaka Y, Band H, Bloom BR, Golan DE, Brenner MB: Direct presentation of nonpeptide prenyl pyrophosphate antigens to human gamma delta T cells. *Immunity* 1995;3:495–507.
- 48 Deusch K, Luling F, Reich K, Classen M, Wagner H, Pfeffer K: A major fraction of human intraepithelial lymphocytes simultaneously expresses the $\gamma\delta$ cell receptor, the CD8 accessory molecule and preferentially uses the V δ 1 gene segment. *Eur J Immunol* 1991;21: 1053–1059.
- 49 Halstensen TS, Scott H, Brandtzaeg P: Intraepithelial T cells of the TcR $\gamma\delta$ ⁺ CD8[−] and V δ 1/J δ 1⁺ phenotypes are increased in coeliac disease. *Scand J Immunol* 1989;30:665–672.
- 50 Spencer J, Isaacson PG, MacDonald TT, Thomas AJ, Walker-Smith JA: Gamma/delta T cells and the diagnosis of coeliac disease. *Clin Exp Immunol* 1991;85:109–113.
- 51 Bucht A, Söderström K, Esin S, Grunewald J, Hagelberg S, Magnusson I, Wigzell H, Grönberg A, Kiessling R: Analysis of $\gamma\delta$ V region usage in normal and diseased human intestinal biopsies and peripheral blood by polymerase chain reaction (PCR) and flow cytometry. *Clin Exp Immunol* 1995;99:57–64.
- 52 McVay LD, Li B, Biancanello R, Creighton MA, Bachwich D, Lichtenstein G, Rombeau JL, Carding SR: Changes in human mucosal $\gamma\delta$ T cell repertoire and function associated with the disease process in inflammatory bowel disease. *Mol Med* 1997;3:183–203.
- 53 Groh V, Steinle A, Bauer S, Spies T: Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells. *Science* 1998;279: 1737–1740.
- 54 Griffith E, Ramsburg E, Hayday A: Recognition by human gut gamma delta cells of stress inducible major histocompatibility molecules on enterocytes. *Gut* 1998;43:166–167.
- 55 Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T: Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci USA* 1996;93:12445–12450.
- 56 Li P, Willie S, Bauer S, Morris DL, Spies T, Strong RK: Crystal structure of the MHC class homolog MIC-A, a $\gamma\delta$ T cell ligand. *Immunity* 1999;10:577–584.
- 57 Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T: Broad tumor-associated expression and recognition by tumor-derived $\gamma\delta$ T cells of MICA and MICB. *Proc Natl Acad Sci USA* 1999;96:6879–6884.
- 58 Vincent MS, Roessner K, Lynch D, Wilson D, Cooper SM, Tschopp J, Sigal SH, Budd RC: Apoptosis of Fas^{high} CD4⁺ synovial T cells by *Borrelia-reactive* Fas-ligand^{high} $\gamma\delta$ T cells in Lyme arthritis. *J Exp Med* 1996;184:2109–2117.
- 59 Vincent MS, Roessner K, Sellati T, Huston CD, Sigal LH, Behar SM, Radolf JD, Budd RC: Lyme arthritis synovial $\gamma\delta$ T cells respond to *Borrelia burgdorferi* lipoproteins and lipidated hexapeptides. *J Immunol* 1998;161:5762–5771.
- 60 Born W, Hall L, Dallas A, Boymel J, Shinnick T, Young D, Brennan P, O'Brien R: Recognition of a peptide antigen by heat shock reactive $\gamma\delta$ T lymphocytes. *Science* 1990;249:67–69.

- 61 Haregewoin A, Singh B, Gupta RS, Finberg RW: A mycobacterial heat shock protein responsive $\gamma\delta$ T cell clone also responds to the homologous human heat shock protein: A possible link between infection and autoimmunity. *J Infect Dis* 1990;163:156–160.
- 62 Laad AD, Thomas ML, Fakhri AR, Chiplunkar SV: Human gamma delta T cells recognize heat shock protein-60 on oral tumor cells. *Int J Cancer* 1999;80:709–714.
- 63 Holoshitz J, Vila LM, Keroack BJ, McKinley DR, Bayne NK: Dual antigenic recognition by cloned human gamma delta T cells. *J Clin Invest* 1992;89:308–314.
- 64 Pao W, Wen L, Smith AL, Gulbranson-Judge A, Zheng B, Kelsoe G, MacLennan ICM, Owen MJ, Hayday AC: $\gamma\delta$ T cell help of B cells is induced by repeated parasitic infection, in the absence of other T cells. *Curr Biol* 1996;6:1317–1325.
- 65 Szczepanik M, Nowak B, Askenase PW, Ptak W: Cross-talk between gamma delta T lymphocytes and immune cells in humoral response. *Immunology* 1998;95:612–617.
- 66 Skeen MJ, Ziegler HK: Intercellular interactions and cytokine responsiveness of peritoneal $\alpha\beta$ and $\gamma\delta$ T cells from *Listeria*-infected mice: Synergistic effects of interleukin 1 and 7 on $\gamma\delta$ T cells. *J Exp Med* 1993;178:985–996.
- 67 Dieli F, Ptak W, Sireci G, Romano GC, Potesio M, Salerno A, Asherson GL: Cross-talk between V beta 8+ and gamma delta+ T lymphocytes in contact sensitivity. *Immunology* 1998;93:469–477.
- 68 Stinissen P, Zhang J, Vandevyver C, Hermans G, Raus J: Gamma delta T cell responses to activated T cells in multiple sclerosis patients induced by T cell vaccination. *J Neuroimmunol* 1998;87:94–104.
- 69 Collins RA, Werling D, Duggan SE, Bland AP, Parsons KR, Howard CJ: $\gamma\delta$ T cells present antigen to CD4+ $\alpha\beta$ T cells. *J Leukoc Biol* 1998;63:707–714.
- 70 Pechhold K, Wesch D, Schöndelmaier S, Kabelitz D: Primary activation of V gamma 9-expressing gamma delta T cells by *Mycobacterium tuberculosis*. Requirement for Th1-type CD4 T cell help and inhibition by IL-10. *J Immunol* 1994;152:4984–4992.
- 71 Wesch D, Marx S, Kabelitz D: Comparative analysis of alpha beta and gamma delta T cell activation by *Mycobacterium tuberculosis* and isopentenyl pyrophosphate. *Eur J Immunol* 1997;27:952–956.
- 72 Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P, Pretolani M: Requirement for $\gamma\delta$ T cells in allergic airway inflammation. *Science* 1998;280:1265–1267.
- 73 Lahn M, Kanehio A, Takeda K, Joetham A, Schwarze J, Köhler G, O'Brien R, Gelfand EW, Born W: Negative regulation of airway responsiveness that is dependent on $\gamma\delta$ T cells and independent of $\alpha\beta$ T cells. *Nat Med* 1999;5:1150–1156.

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