and anergy to non-peptidic mycobacterial antigens in asyptomatic HIV-1 infected persons. *J. Immunol. (in press)*.

Porcelli, S. *et al.* (1989), Recognition of cluster of differentiation 1 antigens by human $CD4^-CD8^-$ cytolytic T lymphocytes. *Nature* (Lond.), 341, 447-449.

Puzo, G. (1990), The carbohydrate and lipid containing cell wall of mycobacteria. *Crit. Rev. Microbiol.*, 17, 305-327.

Roussilhon, C., Agrapart, M., Ballet, J.J. & Bensussan, A. (1990), T lymphocytes bearing the γδ T cell receptor in patients with acute *Plasmodium falciparum* malaria. *J. Infect. Dis.*, 162, 283-285.

Rust, C.J.J., Verreck, F., Vietor, H. & Koning, F. (1990), Specific recognition of staphylococcal enterotoxin A by human T cells bearing the Vγ9 region. *Nature* (Lond.), 346, 572-574.

Scalise, F. *et al.* (1992), Lymphocytes bearing the gamma delta T-cell receptor in acute toxoplasmosis. *Immunology*, 76, 668-70.

Schoel, B., Sprenger, S. & Kaufmann, S.H. (1994), Phosphate is essential for stimulation of V gamma 9V delta 2 T lymphocytes by mycobacterial low molecular weight ligand. *Eur. J. Immunol.*, 24, 1886-92.

Selin, L. K. *et al.* (1992), Reactivity of gamma delta T cells induced by the tumour cell line RPMI 8226: functional heterogeneity of clonal populations and role of GroEL heat shock proteins. *Scand. J. Immunol.*, 36, 107-17.

Sumida, T. *et al.* (1992), Predominant expansion of V gamma 9/V delta 2 T cells in a tularemia patient. *Infect. Immun.*, 60, 2554-8.

Tanaka, Y. *et al.* (1995), Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature* (Lond.), 375, 155-158.

Tanaka, Y. *et al.* (1994), Nonpeptide ligands for human gamma delta T cells. *Proc. Natl. Acad. Sci.* USA, 91, 8175-9.

Tsukaguchi, K., Balaji, K.N. & Boom, W.H. (1995), $CD4^+$ αβ T cell and γδ T cell responses to *Mycobacterium tuberculosis*. Similarities and differences in Ag recognition, cytotoxic effector function, and cytokine production. *J. Immunol.*, 154, 1786-1796.

Ueta, C. *et al.* (1994), Increase of gamma/delta T cells in hospital workers who are in close contact with tuberculosis patients. *Infect. Immun.*, 62, 5434-41.

Uyemura, K. *et al.* (1991), Evidence for clonal selection of gamma/delta T-cells in response to a human pathogen. *J. Exp. Med.*, 174, 683-692.

Uyemura, K. *et al.* (1992), Selective expansion of $V\delta1^+$ T cells from leprosy skin lesions. *J. Invest. Dermatol.*, 99, 848-852.

Wallace, M., Malkovski, M. & Carding, S.R. (1995), Gamma/delta T lymphocytes in viral infections. *J. Leuko. Biol.*, 58, 277-283.

Young, J.L. & Gaston, J.S.H. (1995), $\gamma\delta^+$ T cell reactive against *Yersinia enterocolitica* display MHC-independant cytotoxicity. The 9th International Congress of Immunology, San Francisco, California, USA.

Direct presentation of non-peptide prenyl pyrophosphate antigens to human γδ T cells

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Introduction

T cells can be divided into two subsets based on their expression of αβ or γδ T-cell antigen receptors. Although γδ T cells were discovered 10 years ago (Brenner *et al.*, 1986), relatively little is known about the types of antigens that stimulate γδ T cells or the presentation elements and presentation path-

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ways that these antigens use. Few γδ T cells respond to conventional peptide antigens or superantigens presented by MHC class I or class II molecules that stimulate αβ T cells (Haas *et al.*, 1993; Porcelli *et al.*, 1991).

There were many indications that antigen recognition by γδ T cells might be very different from antigen recognition by αβ T cells. Although human αβ and γδ T cells share a similar structure for their antigen receptors, a variable heterodimer that associates with the invariant CD3 signalling complex, there are greater than 50 Vα and 46 Vβ gene segments that can pair to make several thousand receptor combinations. Any one pair constitutes only a small percentage of the blood T cells. In stark contrast, there are only 6 Vγ and 3-4 major Vδ gene segments used by human γδ T cells (Porcelli *et al.*, 1991). Moreover, in normal adults, one of these pairs, the Vγ2Vδ2 T-cell receptor, predominates, being expressed on approximately 50-75% of the γδ T cells. Thus, this one TCR pair is present on 3-5% of human T cells (Morita *et al.*, 1994; Parker *et al.*, 1990). A similar restricted repertoire of Vγ and Vδ gene segments is seen in rodents (Haas *et al.*, 1993).

αβ and γδ T cells also differ in their functional capabilities. In general, αβ T cells can be divided into helper cells that express CD4 or killer cells that express CD8. In contrast, the majority of γδ T cells are killer cells that express neither CD4 nor CD8 (Groh *et al.*, 1989; Morita *et al.*, 1991). Like αβ T cells, γδ T cells secrete a variety of cytokines, although they make somewhat lesser amounts of IL2 and GM-CSF (Morita *et al.*, 1991; Patel *et al.*, 1989; Spits *et al.*, 1991). Thus, given the differences in the TCR repertoire and functional capabilities, antigen recognition by γδ T cells might be distinct from antigen recognition by αβ T cells.

Recently, we have identified non-peptide prenyl pyrophosphate antigens that stimulate the major subset of human γδ T cells (Tanaka *et al.*, 1995). This has allowed us to study the presentation pathways used by these novel antigens. We find that γδ T cells recognize an entirely new class of antigens through an extracellular presentation pathway that does not require antigen uptake, antigen processing, or known antigen-presenting elements (Morita *et al.*, 1995).

Human γδ T cells and infections

One of the first suggestions that human γδ T cells could recognize foreign antigens was from studies examining the development of the γδ T-cell repertoire (Parker *et al.*, 1990). γδ T-cell numbers in the peripheral blood increase between ages 1-10. This peripheral expansion of γδ T cells is restricted to the Vγ2Vδ2 subset and leads to the predominance of this subset in the γδ T-cell repertoire of most adults and the high level of $V\gamma2V\delta2^+$ T cells found among peripheral blood T cells. Identical twins were found that were disparate for the level of expression of the Vγ2Vδ2 T-cell subset in that one twin had high levels of Vγ2Vδ2 T cells while the other did not. As identical twins are genetically identical, environmental factors appear to influence the development of the γδ T-cell repertoire.

Infections with microorganisms are likely candidates for environmental factors that influence γδ T-cell development. γδ T cells were found to increase dramatically in the peripheral blood in response to a variety of infections, both bacterial (Balbi *et al.*, 1993; Bertotto *et al.*, 1993; Caldwell *et al.*, 1995; Hara *et al.*, 1992; Sumida *et al.*, 1992; Ueta *et al.*, 1994) and protozoal (Ho *et al.*, 1990; Perera *et al.*, 1994; Scalise *et al.*, 1992) (table I). In patients with infections with *Ehrlichia,* a rickettsial organism, extremely high levels of γδ T cells were observed (up to 97% of peripheral blood T cells) (Caldwell *et al.*, 1995). Moreover, these γδ expansions are limited to cells expressing the major Vγ2Vδ2 T-cell receptor.

These *in vivo* expansions of γδ T cells can be reproduced *in vitro* by exposure of normal peripheral blood mononuclear cells to extracts from mycobacteria (De Libero *et al.*, 1991; Kabelitz *et al.*, 1990; Panchamoorthy *et al.*, 1991). Like the *in vivo* expansions, the *in vitro* expansions were limited to cells bearing the Vγ2Vδ2 T-cell receptor (De Libero *et al.*, 1991; Kabelitz *et al.*, 1991). Upon sequencing of the Vγ2Vδ2 T-cell antigen receptors, these expanded populations were found to be polyclonal (Ohmen *et al.*, 1991; Panchamoorthy *et al.*, 1991). This suggested that germline-encoded segments of the T-cell receptor were involved in the recognition of soluble antigens from mycobacteria.

γδ T-cell clones expressing the Vγ2Vδ2 T-cell receptor also responded to soluble extracts from *Mycobacterium tuberculosis in vitro* (De Libero *et al.*, 1991; Holoshitz *et al.*, 1989). This allowed us to test whether one γδ T cell could respond to different pathogens or whether distinct populations of Vγ2Vδ2 T cells were responding to different pathogens. Vγ2Vδ2 T-cell clones responded to extracts from a number of different bacteria including mycobacteria, Gram-positive and -negative rods and Gram-positive cocci (data not shown). Therefore, individual Vγ2Vδ2 T cells can respond to a number of different microorganisms, suggesting that Vγ2Vδ2 T cells may recognize common structural features of antigens from the microorganisms.

Table I. Expansion of human γδ T cells in response to infection.

	Disease	γδ T cells (mean % of CD3+) Normals	Patients (max)	Reference
Bacterial infections	Salmonellosis	5	18 (48)	(Hara *et al.*, 1992)
	Tularaemia	3	33 (33)	(Sumida *et al.*, 1992)
	Brucellosis	4	29 (48)	(Bertotto *et al.*, 1993)
	Tuberculosis	6	14 (35)	(Balbi *et al.*, 1993)
	Tuberculosis contacts	5	10 (18)	(Ueta *et al.*, 1994)
	Ehrlichiosis	5	57 (97)	(Caldwell *et al.*, 1995)
Protozoal infections	Visceral leishmaniasis	8	44	(Raziuddin *et al.*, 1992)
	Acute malaria	4	16 (27)	(Ho *et al.*, 1990)
	Malarial paroxysms	4	11 (27)	(Perera *et al.*, 1994)
	Toxoplasmosis	4	9 (15)	(Scalise *et al.*, 1992)

Isopentenyl pyrophosphate and related non-peptide compounds from mycobacteria are antigens for human Vγ2Vδ2+ T cells

To help determine the molecular basis for the recognition of mycobacterial antigens, we isolated and identified two antigens from mycobacteria that stimulate γδ T cells. Preliminary studies by Pfeffer *et al.* demonstrated that these antigens were protease-resistant and extremely small, suggesting that the antigens for γδ T cells are non-proteinaceous compounds (Pfeffer *et al.*, 1990; 1992). Subsequently, these antigens were shown to contain critical phosphate residues on the basis of their sensitivity to alkaline phosphatase and nucleotide pyrophosphatase (Constant *et al.*, 1994; Schoel *et al.*, 1994; Tanaka *et al.*, 1994).

Based on these and other experiments, we devised a purification protocol to isolate these antigens from *Mycobacteria smegmatis*. Two antigens were purified and identified by their fragmentation spectra on tandem mass spectroscopy (Tanaka *et al.*, 1995). Isopentenyl pyrophosphate (fig. 1) and its related hydroxymethyl derivative were able to stimulate Vγ2Vδ2 T-cell clones and to expand the Vγ2Vδ2 subset from peripheral blood cells. These are among the first, natural, non-peptide antigens described for T cells and constitute a new class of antigens distinct from peptide antigens, superantigens, and the recently described lipid antigens.

Isopentenyl pyrophosphate has two major structural features; a carbon isoprenyl chain and a pyrophosphate moiety. Studies with structural analogs of isopentenyl pyrophosphate demonstrate that Vγ2Vδ2 T cells show specificity in their recognition of phosphate-containing compounds and can only recognize non-bulky carbon chains that do not contain amino groups (Bürk *et al.*, 1995; Tanaka *et al.*, 1995; 1994) and data not shown). Similarly, changes in the pyrophosphate moiety also affect recognition of isopentenyl pyrophosphate. Multiple substitutions of the phosphate group were not permitted. Removal of a phosphate group from isopentenyl pyrophosphate decreased specific biological activity by 230-fold. The addition of a uridine triphosphate molecule had minimal effects on recognition.

The addition of a nucleotide triphosphate group was suggested from the results of Constant *et al* (Constant *et al.*, 1994) who have described a TTP-containing antigen (TUBag4) for Vγ2Vδ2 T cells from the cytoplasm of *M. tuberculosis,* although they have not as yet determined the structure of the carbon chain. They also find two pyrophosphate-containing compounds, TUBag1 and TUBag2, but have not yet determined the structure of the carbon chain. Thus, there may be a number of pyrophosphate-containing compounds produced by different microorganisms.

The identification of isopentenyl pyrophosphate and related compounds as natural antigens from mycobacteria for Vγ2Vδ2+ T cells suggests an explanation as to why γδ T cells respond to so many different types of pathogens. Isopentenyl pyrophosphate is an essential precursor for a variety of biological molecules such as cholesterol and its derivatives, vitamins, dolicol phosphates and ubiquinones, and is required for the membrane anchoring of a number of important signal transduction molecules such as ras. As both prokaryotic and eukaryotic

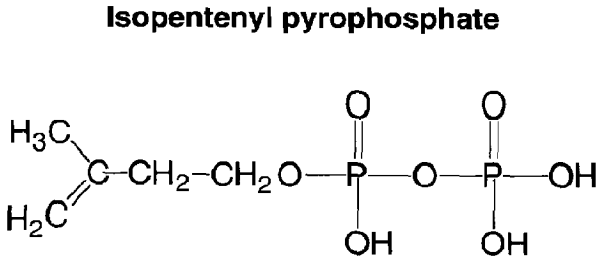

Fig. 1. Structure of isopentenyl pyrophosphate.

There are two main structural features, the isopentenyl carbon chain (on left) and the pyrophosphate moiety (on right).

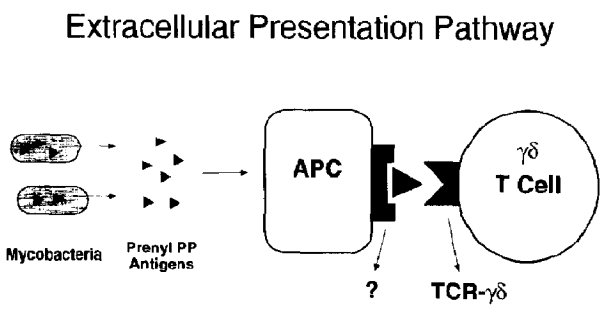

Fig. 2. Proposed extracellular presentation pathway for the prenyl pyrophosphate antigens.

organisms use isopentenyl pyrophosphate and related compounds as essential precursors, Vγ2Vδ2-bearing T cells can respond to infection by both prokaryotic and eukaryotic pathogens. Given the variety of different microorganisms that stimulate Vγ2Vδ2-bearing T cells, the number of such prenyl pyrophosphate derivatives recognized by γδ T cells may be expected to grow in coming years.

Extracellular presentation pathway for the prenyl pyrophosphate antigens

Our identification of prenyl pyrophosphate compounds as antigens for Vγ2Vδ2 T cells allowed us to examine the characteristics of the presentation of these antigens and to investigate the involvement of known antigen presenting molecules in recognition (Morita *et al.*, 1995).

Recognition of the non-peptide prenyl pyrophosphate antigens is restricted to γδ T-cell clones expressing Vγ2Vδ2 T-cell receptors (Davodeau *et al.*, 1993; Tanaka *et al.*, 1994). This recognition is TCR-dependent as cDNAs encoding Vγ2 and Vδ2 chains confer non-peptide antigen recognition when transfected into a TCR-negative Jurkat T-cell tumour mutant line (Bukowski *et al.*, 1995). The Vγ2Vδ2 transfectant, DBS43, released IL2 in response to isopentenyl pyrophosphate but not in response to the biologically inactive compound, phenethylpyrophosphate (Tanaka *et al.*, 1995). A control Vγ1Vδ1 TCR transfectant did not respond to either compound, although it did respond to mAbs directed to the TCR. These results establish the TCR dependency of the response to the prenyl pyrophosphate antigens.

Presentation of the prenyl pyrophosphate antigens has unique features in comparison to the presentation of protein antigens (Morita *et al.*, 1995). Mycobacteria are able to secrete non-peptide antigens, suggesting that γδ T cells may encounter free antigen *in vivo*. Professional antigen-presenting cells are not required for the presentation of prenyl pyrophosphate antigens (Morita *et al.*, 1995; Vila *et al.*, 1995). The majority of Vγ2Vδ2-bearing T-cell clones and the Vγ2Vδ2 TCR transfectant could respond to non-peptide antigens in the absence of additional APCs. Moreover, recognition of non-peptide antigens is rapid, occurring as soon as 2-3 min. after their addition (Morita *et al.*, 1995). This suggests that antigen uptake and processing are not required and consistent with this hypothesis, both non-fixed and fixed APC can present non-peptide prenyl pyrophosphate antigens to an APC-dependent γδ T-cell clone (Fisch *et al.*, 1990; Morita *et al.*, 1995). Thus, fixed APC which are unable to take up antigens or process them could still present the prenyl pyrophosphate antigens.

The presentation of the prenyl pyrophosphate antigens most resembles the presentation of preprocessed peptide antigens to human αβ T cells. Peptide antigens can stimulate most reactive αβ T cells in the absence of APC and antigen uptake and processing because the peptides can bind directly to MHC class I or class II molecules on the T-cell surface. The resulting MHC/peptide complexes are then presented to neighbouring T cells (Pichler and Wyss-Coray, 1994). This presentation of peptides by one T cell to another requires cell-cell contact. A similar cell-cell contact requirement is observed for the recognition of the prenyl pyrophosphate antigens, as was determined by increases in calcium flux (Morita *et al.*, 1995) and in TNFα production (Lang *et al.*, 1995) in response to exposure to the non-peptide antigens.

Even though the presentation of the prenyl pyrophosphate antigens required cell-cell contact, known antigen-presenting molecules were not required for their recognition (Fisch *et al.*, 1990; Holoshitz *et al.*, 1993; Morita *et al.*, 1995). Monoclonal antibodies directed to class II, class I, and CD1 molecules did not block the recognition of the prenyl pyrophosphate antigens (Morita *et al.*, 1995). Moreover, the

Vγ2Vδ2 TCR transfectant lacked MHC class II expression but was able to respond to prenyl pyrophosphate antigens in the absence of APC (Morita *et al.*, 1995). Lastly, mutant APC lacking classical class I, β_2m, or class II molecules were as efficient as wild type APC in presenting mycobacterial non-peptide antigens to an APC-dependent CD4$^+$ γδ T-cell clone and class II-negative APC could function as targets for γδ T cells in the presence of antigens from *M. tuberculosis* (Morita *et al.*, 1995). Therefore, the presentation of the prenyl pyrophosphate antigens does not involve known antigen-presenting molecules.

It is not clear if a novel antigen-presenting element exists for the non-peptide prenyl pyrophosphate antigens. Unlike most peptide antigens and superantigens, culturing antigen-presenting cells with the prenyl pyrophosphate antigens does not render the "pulsed" antigen presenting cells stimulatory for Vγ2Vδ2 bearing T cells (Lang *et al.*, 1995; Morita *et al.*, 1995). One interpretation of this result is that non-peptide antigens do not stably associate with the antigen-presenting element on the surface of the APC. A similar lack of stable association between peptides and the murine MHC class II allele, I-A^{g7}, has been reported whereby immunogenic peptides cannot be pulsed onto the APC surface although the peptides stimulate I-A^{g7}-restricted T cells when cultured together (Carrasco-Marin *et al.*, 1996). The putative presenting element would be predicted to be non-polymorphic and to be widely expressed on many types of cells.

Thus, we propose the following model for the extracellular presentation of prenyl pyrophosphate antigens. The natural antigens for Vγ2Vδ2-bearing T cells are isopentenyl pyrophosphate and related prenyl pyrophosphate compounds. These compounds are synthesized by mycobacteria and a variety of other bacteria, as they are essential metabolites for all living things. However, mycobacteria can secrete these compounds extracellularly where they can associate with the surface of an antigen-presenting cell, perhaps by binding to a novel antigen-presenting element. The T-cell receptor of Vγ2Vδ2 T cells can recognize these compounds. The T cells then become activated to secrete cytokines, proliferate, and kill neighbouring cells. This process requires T cell/APC cell-cell contact but does not require that the APC be a professional APC, nor does it require antigen uptake or processing. In this respect, the presentation of the non-peptide prenyl pyrophosphate antigens is quite distinct from the presentation of conventional protein antigens and superantigens by MHC molecules or lipid antigens by CD1 molecules.

This novel extracellular pathway allows for the direct and rapid recognition of prenyl pyrophosphate antigens by γδ T cells. This suggests that γδ T-cell recognition non-peptide antigen might complement αβ T-cell antigen recognition of protein antigens that are presented by intracellular pathways. In this way, γδ T cells might function as a bridge between the innate immunity of myeloid cells, NK cells and complement and the adaptive immunity of αβ T cells and B cells. By recognizing a new class of non-peptide antigens, γδ T cells probably play important and unique roles in human immunity and autoimmunity.

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References

Balbi, B., Valle, M.T., Oddera, S., Giunti, D., Manca, F., Rossi, G.A. & Allegra, L. (1993), T-lymphocytes with γδ$^+$ Vδ2$^+$ antigen receptors are present in increased proportions in a fraction of patients with tuberculosis or with sarcoidosis. *Am. Rev. Respir. Dis.*, 148, 1685-1690.

Bertotto, A., Gerli, R., Spinozzi, F., Muscat, C., Scalise, F., Castellucci, G., Sposito, M., Candio, F. & Vaccaro, R. (1993), Lymphocytes bearing the γδ T cell receptor in acute *Brucella melitensis* infection. *Eur. J. Immunol.*, 23, 1177-1180.

Brenner, M.B., McLean, J., Dialynas, D.P., Strominger, J.L., Smith, J.A., Owen, F.L., Seidman, J.G., Ip, S., Rosen, F. & Krangel, M.S. (1986), Identification of a putative second T-cell receptor. *Nature* (Lond.), 322, 145-149.

Bukowski, J.F., Morita, C.T., Tanaka, Y., Bloom, B.R., Brenner, M.B. & Band, H. (1995), Vγ2Vδ2 TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. *J. Immunol.*, 154, 998-1006.

Bürk, M.R., Mori, L. & De Libero, G. (1995), Human Vγ9-Vδ2 cells are stimulated in a cross-reactive fashion by a variety of phosphorylated metabolites. *Eur. J. Immunol.*, 25, 2052-2058.

Caldwell, C.W., Everett, E.D., McDonald, G., Yesus, Y.W. & Roland, W.E. (1995), Lymphocytosis of γ/δ T cells in human ehrlichiosis. *Am. J. Clin. Path.*, 103, 761-766.

Carrasco-Marin, E., Shimizu, J., Kanagawa, O. & Unanue, E.R. (1996), The class II MHC I-A^{g7} molecules from non-obese diabetic mice are poor peptide binders. *J. Immunol.*, 156, 450-458.

Constant, P., Davodeau, F., Peyrat, M.-A., Poquet, Y., Puzo, G., Bonneville, M. & Fournié, J.-J. (1994), Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands. *Science*, 264, 267-270.

Davodeau, F., Peyrat, M.-A., Hallet, M.-M., Gaschet, J., Houde, I., Vivien, R., Vie, H. & Bonneville, M. (1993), Close correlation between Daudi and mycobacterial antigen recognition by human γδ T cells and expression of V9JPC1γ/V2DJCδ-encoded T cell receptors. *J. Immunol.*, 151, 1214-1223.

De Libero, G., Casorati, G., Giachino, C., Carbonara, C.,

Migone, N., Matzinger, P. & Lanzavecchia, A. (1991), Selection by two powerful antigens may account for the presence of the major population of human peripheral γ/δ T cells. *J. Exp. Med.*, 173, 1311-1322.

Fisch, P., Malkovsky, M., Kovats, S., Sturm, E., Braakman, E., Klein, B.S., Voss, S.D., Morrissey, L.W., DeMars, R., Welch, W.J., Bolhuis, R.L.H. & Sondel, P.M. (1990), Recognition by human Vγ9/Vδ2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. *Science*, 250, 1269-1273.

Groh, V., Porcelli, S., Fabbi, M., Lanier, L.L., Picker, L.J., Anderson, T., Warnke, R.A., Bhan, A.K., Strominger, J.L. & Brenner, M.B. (1989), Human lymphocytes bearing T cell receptor γ/δ are phenotypically diverse and evenly distributed throughout the lymphoid system. *J. Exp. Med.*, 169, 1277-1294.

Haas, W., Pereira, P. & Tonegawa, S. (1993), Gamma/delta cells. *Annu. Rev. Immunol.*, 11, 637-685.

Hara, T., Mizuno, Y., Takaki, K., Takada, H., Akeda, H., Aoki, T., Nagata, M., Ueda, K., Matsuzaki, G., Yoshikai, Y. & Nomoto, K. (1992), Predominant activation and expansion of Vγ9-bearing γδ T cells in vivo as well as in vitro in *Salmonella* infection. *J. Clin. Invest.*, 90, 204-210.

Ho, M., Webster, H.K., Tongtawe, P., Pattanapanyasat, K. & Weidanz, W.P. (1990), Increased γδ T cells in acute *Plasmodium falciparum* malaria. *Immunol. Lett.*, 25, 139-142.

Holoshitz, J., Koning, F., Coligan, J.E., De Bruyn, J. & Strober, S. (1989), Isolation of CD4⁻ CD8⁻ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature* (Lond.), 339, 226-229.

Holoshitz, J., Romzek, N.C., Jia, Y., Wagner, L., Vila, L.M., Chen, S.-J., Wilson, J.M. & Karp, D.R. (1993), MHC-independent presentation of mycobacteria to human γδ T cells. *Int. Immunol.*, 5, 1437-1443.

Kabelitz, D., Bender, A., Prospero, T., Wesselborg, S., Janssen, O. & Pechhold, K. (1991), The primary response of human γ/δ⁺ T cells to *Mycobacterium tuberculosis* is restricted to Vγ9-bearing cells. *J. Exp. Med.*, 173, 1331-1338.

Kabelitz, D., Bender, A., Schondelmaier, S., Schoel, B. & Kaufmann, S.H.E. (1990), A large fraction of human peripheral blood γ/δ⁺ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. *J. Exp. Med.*, 171, 667-679.

Lang, F., Peyrat, M.A., Constant, P., Davodeau, F., David-Ameline, J., Poquet, Y., Vié, H., Fournié, J.J. & Bonneville, M. (1995), Early activation of human Vγ9Vδ2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. *J. Immunol.*, 154, 5986-5994.

Morita, C.T., Beckman, E.M., Bukowski, J.F., Tanaka, Y., Band, H., Bloom, B.R., Golan, D.E. & Brenner, M.B. (1995), Direct presentation of nonpeptide prenyl pyrophosphate antigens to human γδ T cells. *Immunity*, 3, 495-507.

Morita, C.T., Parker, C.M., Brenner, M.B. & Band, H. (1994), T cell receptor usage and functional capabilities of human γδ T cells at birth. *J. Immunol.*, 153, 3979-3988.

Morita, C.T., Verma, S., Aparicio, P., Martinez-A., C., Spits, H. & Brenner, M.B. (1991), Functionally distinct subsets of human γ/δ T cells. *Eur. J. Immunol.*, 21, 2999-3007.

Ohmen, J.D., Barnes, P.F., Uyemura, K., Lu, S.Z., Grisso, C.L. & Modlin, R.L. (1991), The T cell receptors of human γδ T cells reactive to *Mycobacterium tuberculosis* are encoded by specific V genes but diverse V-J junctions. *J. Immunol.*, 147, 3353-3359.

Panchamoorthy, G., McLean, J., Modlin, R.L., Morita, C.T., Ishikawa, S., Brenner, M.B. & Band, H. (1991), A predominance of the T cell receptor Vγ2/Vδ2 subset in human mycobacteria-responsive T cells suggests germline gene encoded recognition. *J. Immunol.*, 147, 3360-3369.

Parker, C.M., Groh, V., Band, H., Porcelli, S.A., Morita, C., Fabbi, M., Glass, D., Strominger, J.L. & Brenner, M.B. (1990), Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J. Exp. Med.*, 171, 1597-1612.

Patel, S.S., Wacholtz, M.C., Duby, A.D., Thiele, D.L. & Lipsky, P.E. (1989), Analysis of the functional capabilities of CD3⁺CD4⁻CD8⁻ and CD3⁺CD4⁺ CD8⁺ human T cell clones. *J. Immunol.*, 143, 1108-1117.

Perera, M.K., Carter, R., Goonewardene, R. & Mendis, K.N. (1994), Transient increase in circulating γ/δ T cells during *Plasmodium vivax* malarial paroxysms. *J. Exp. Med.*, 179, 311-315.

Pfeffer, K., Schoel, B., Gulle, H., Kaufmann, S.H.E. & Wagner, H. (1990), Primary responses of human T cells to mycobacteria: a frequent set of γ/δ T cells are stimulated by protease-resistant ligands. *Eur. J. Immunol.*, 20, 1175-1179.

Pfeffer, K., Schoel, B., Plesnila, N., Lipford, G.B., Kromer, S., Deusch, K. & Wagner, H. (1992), A lectin-binding, protease-resistant mycobacterial ligand specifically activates Vγ9⁺ human γδ T cells. *J. Immunol.*, 148, 575-583.

Pichler, W.J. & Wyss-Coray, T. (1994), T cells as antigen-presenting cells. *Immunol. Today*, 15, 312-315.

Porcelli, S., Brenner, M.B. & Band, H. (1991), Biology of the human γδ T-cell receptor. *Immunol. Rev.*, 120, 137-183.

Raziuddin, S., Telmasani, A.W., El-Awad, M.E., Al-Amari, O. & Al-Janadi, M. (1992), γδ T cells and the immune response in visceral leishmaniasis. *Eur. J. Immunol.*, 22, 1143-1148.

Scalise, F., Gerli, R., Castellucci, G., Spinozzi, F., Fabietti, G.M., Crupi, S., Sensi, L., Britta, R., Vaccaro, R. & Bertotto, A. (1992), Lymphocytes bearing the γδ T-cell receptor in acute toxoplasmosis. *Immunology*, 76, 668-670.

Schoel, B., Sprenger, S. & Kaufmann, S.H.E. (1994), Phosphate is essential for stimulation of Vγ9Vδ2 T lymphocytes by mycobacterial low molecular weight ligand. *Eur. J. Immunol.*, 24, 1886-1892.

Spits, H., Paliard, X., Vandekerckhove, Y., van Vlasselaer, P. & de Vries, J.E. (1991), Functional and phenotypic differences between CD4⁺ and CD4⁻ T cell receptor-γδ clones from peripheral blood. *J. Immunol.*, 147, 1180-1188.

Sumida, T., Maeda, T., Takahashi, H., Yoshida, S., Yonaha, F., Sakamoto, A., Tomioka, H., Koike, T. & Yoshida, S. (1992), Predominant expansion of Vγ9/Vδ2 T cells in a tularemia patient. *Infect. Immun.*, 60, 2554-2558.

Tanaka, Y., Morita, C.T., Tanaka, Y., Nieves, E., Brenner, M.B. & Bloom, B.R. (1995), Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature* (Lond.), 375, 155-158.

Tanaka, Y., Sano, S., Nieves, E., De Libero, G., Roca, D., Modlin, R.L., Brenner, M.B., Bloom, B.R. & Morita, C.T. (1994), Nonpeptide ligands for human γδ T cells. *Proc. Natl. Acad. Sci.* USA, 91, 8175-8179.

Ueta, C., Tsuyuguchi, I., Kawasumi, H., Takashima, T., Toba, H. & Kishimoto, S. (1994), Increase of γ/δ T cells in hospital workers who are in close contact with tuberculosis patients. *Infect. Immun.*, 62, 5434-5441.

Vila, L.M., Haftel, H.M., Park, H.-S., Lin, M.-S., Romzek, N.C., Hanash, S.M. & Holoshitz, J. (1995), Expansion of mycobacterium-reactive γδ T cells by a subset of memory helper T cells. *Infect. Immun.*, 63, 1211-1217.

Note added in proofs:

Response of Kasahara *et al.*

We would like to thank Drs. Ahouse and Simister for writing the commentary on our article on the original evolution of the class I genes. We would like to address some of the issues they raised and clarify potential misunderstandings.

First, Ahouse and Simister suggest that structural variations among class I heavy chains are likely to be constrained by the need to always interact with the same partner, β_2m. We agree with this notion. However, we do not agree with the idea that class II molecules can vary more easily than class I molecules because they do need to interact with β_2m. Germain and colleagues (1985) showed that even minor allelic variations can affect the pairing of class II A and B chains. This is in contrast to the fact that the same β_2m molecule can form a heterodimer with structurally divergent heavy chains ranging from those of class Ia to even CD1 molecules. Thus, β_2m is less discriminating than class II A or B chains in choosing its partner, and we believe that, for this reason, class I genes can be more plastic than class II genes.

Second, we are not proposing the existence of a genetic mechanism that selectively translocates class I genes equipped with specialized functions to the outside of the MHC. Because class I genes located outside the MHC class I region are likely to enjoy a longer life span than their MHC-encoded counterparts, they have a better chance of acquiring non-typical functions. We think, therefore, that class I molecules encoded outside the MHC presumably acquired specialized functions as a *consequence* of the translocation.

Finally, the very recent structural analysis of the HSP70 peptide-binding region (Zhu *et al.*, 1996) makes it difficult to justify an evolutionary relationship between class I and HSP70. In order to maintain this hypothesis, one must propose intermediate stages between HSP70 and class I; while these stages may exist (or have existed), we must admit that a gradual evolution of class I from an HSP70 ancestor is not in the spirit of our original model (Flajnik *et al.*, 1991b). Of course the HSP70 structure does not rule out the possibility that it is a forerunner of class I, but the same can be said of other gene families as well — e.g., see Hashimoto and Kurosawa (1991) for a discussion of immunoglobulin domains as MHC ancestors. As suggested by Ahouse and Simister, molecules ancestral to class I may be present in jawless fish and protochordates, taxa in which no evidence exists for *bona fide* MHC. We (and others) will continue to mine for such gems.

References

Germain, R.N., Bentley, D.M. & Quill, H. (1985), Influence of allelic polymorphism on the assembly and surface expression of class II MHC (Ia) molecules. *Cell*, 43, 233-242.

Hashimoto, K. & Kurosawa, Y. (1991), Evolution of MHC domains: strategy for isolation of MHC genes from primitive animals, *in "Molecular Evolution of the Major Histocompatibility Complex"* (J. Klein and D. Klein) (pp. 103-109). Springer-Verlag, Berlin Heidelberg.

Zhu, X., Zhao, X., Burkholder, W.F., Gragerov, A., Ogata, C.M., Gottesman, M.E. & Henderickson, W.A. (1996), Structural analysis of substrate binding by the molecular chaperone DnaK. *Science*, 272, 1606-1614.

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