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-
ways that these antigens use. Few \( \gamma^6 \) T cells respond to conventional peptide antigens or superantigens presented by MHC class I or class II molecules that stimulate \( \alpha\beta \) T cells (Haas et al., 1993; Porcelli et al., 1991).

There were many indications that antigen recognition by \( \gamma^6 \) T cells might be very different from antigen recognition by \( \alpha\beta \) T cells. Although human \( \alpha\beta \) and \( \gamma^6 \) 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 \( V\alpha \) and 46 \( V\beta \) 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 \( V\gamma \) and 3–4 major \( V\delta \) gene segments used by human \( \gamma^6 \) T cells (Porcelli et al., 1991). Moreover, in normal adults, one of these pairs, the \( V\gamma 2V62 \) T-cell receptor, predominates, being expressed on approximately 50–75% of the \( \gamma^6 \) T cells. Thus, this one TCR pair is present on 3–5% of human T cells (Morita et al., 1994, Parkes et al., 1990). A similar restricted repertoire of \( V\gamma \) and \( V\delta \) gene segments is seen in rodents (Haas et al., 1993).

\( \alpha\beta \) and \( \gamma^6 \) T cells also differ in their functional capabilities. In general, \( \alpha\beta \) T cells can be divided into helper cells that express CD4 or killer cells that express CD8. In contrast, the majority of \( \gamma^6 \) T cells are killer cells that express neither CD4 nor CD8 (Groh et al., 1989; Morita et al., 1991). Like \( \alpha\beta \) T cells, \( \gamma^6 \) 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 \( \gamma^6 \) T cells might be distinct from antigen recognition by \( \alpha\beta \) T cells.

Recently, we have identified non-peptide prenyl pyrophosphate antigens that stimulate the major subset of human \( \gamma^6 \) T cells (Tanaka et al., 1995). This has allowed us to study the presentation pathways used by these novel antigens. We find that \( \gamma^6 \) 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 \( \gamma^6 \) T cells and infections

One of the first suggestions that human \( \gamma^6 \) T cells could recognize foreign antigens was from studies examining the development of the \( \gamma^6 \) T-cell repertoire (Parker et al., 1990). \( \gamma^6 \) T-cell numbers in the peripheral blood increase between ages 1–10. This peripheral expansion of \( \gamma^6 \) T cells is restricted to the \( V\gamma 2V62 \) subset and leads to the predominance of this subset in the \( \gamma^6 \) T-cell repertoire of most adults and the high level of \( V\gamma 2V62^{+} \) T cells found among peripheral blood T cells. Identical twins were found that were disparate for the level of expression of the \( V\gamma 2V62 \) T-cell subset in that one twin had high levels of \( V\gamma 2V62 \) T cells while the other did not. As identical twins are genetically identical, environmental factors appear to influence the development of the \( \gamma^6 \) T-cell repertoire.

Infections with microorganisms are likely candidates for environmental factors that influence \( \gamma^6 \) T-cell development. \( \gamma^6 \) 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 1). In patients with infections with *Ehrlichia*, a rickettsial organism, extremely high levels of \( \gamma^6 \) T cells were observed (up to 97% of peripheral blood T cells) (Caldwell et al., 1995). Moreover, these \( \gamma^6 \) expansions are limited to cells expressing the major \( V\gamma 2V62 \) T-cell receptor.

These in vivo expansions of \( \gamma^6 \) 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 \( V\gamma 2V62 \) T-cell receptor (De Libero et al., 1991; Kabelitz et al., 1991). Upon sequencing of the \( V\gamma 2V62 \) 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.

\( \gamma^6 \) T-cell clones expressing the \( V\gamma 2V62 \) 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 \( \gamma^6 \) T cell could respond to different pathogens or whether distinct populations of \( V\gamma 2V62 \) T cells were responding to different pathogens. \( V\gamma 2V62 \) 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 \( V\gamma 2V62 \) T cells can respond to a number of different microorganisms, suggesting that \( V\gamma 2V62 \) T cells may recognize common structural features of antigens from the microorganisms.
Isopentenyl pyrophosphate and related non-peptide compounds from mycobacteria are antigens for human γδ 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 γδ T-cell clones and to expand the γδ T 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 γδ 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 γδ 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 γδ 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, dolichol 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
Isopentenyl pyrophosphate

\[
\text{H}_3\text{C} \quad \text{O} \quad \text{CH}_2\text{-CH}_2\text{O} \quad \text{P} \quad \text{O} \quad \text{OH} \\
\text{H}_2\text{C} \quad \text{O} \quad \text{CH}_2\text{-CH}_2\text{O} \quad \text{P} \quad \text{O} \quad \text{OH}
\]

Fig. 1. Structure of isopentenyl pyrophosphate.

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

Extracellular Presentation Pathway

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 (Hukowski et al., 1995). The Vγ1Vδ1 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 γδ T cells. Peptide antigens can stimulate most reactive γδ 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 (Piclier 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, β2m, 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 Mycobacterium 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-Aβ7, has been reported whereby immunogenic peptides cannot be pulsed onto the APC surface although the peptides stimulate I-Aβ7-restricted T cells when cultured together (Carrasco-Marín 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 neighboring 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.

Acknowledgments

This work was supported by grants from the NIH, the Arthritis Foundation (American College of Rheumatology), and the Howard Hughes Medical Institute.

References


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Notes 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, β2m. 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 not need to interact with β2m. 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 β2m molecule can form a heterodimer with structurally divergent heavy chains ranging from those of class Ia to even CD1 molecules. Thus, β2m 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 protocordates, taxa in which no evidence exists for bona fide MHC. We (and others) will continue to mine for such gems.

References


Key-words: MHC, CD1, Antigen presentation; Non-classical MHC, MHC class I b, MHC evolution; Forum.