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Evolution and Function of the TCR Vgamma9 Chain Repertoire: It's Good to be Public

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Abstract

Lymphocytes expressing a T cell receptor (TCR) composed of Vgamma9 and Vdelta2 chains represent a minor fraction of human thymocytes. Extrathymic selection throughout post-natal life causes the proportion of cells with a Vgamma9-JP rearrangement to increase and elevates the capacity for responding to non-peptidic phosphoantigens. Extrathymic selection is so powerful that phosphoantigen-reactive cells comprise about 1 in 40 circulating memory T cells from healthy adults and the subset can be expanded rapidly upon infection or in response to malignancy. Skewing of the gamma delta TCR repertoire is accompanied by selection for public gamma chain sequences such that many unrelated individuals overlap extensive in their circulating repertoire. This type of selection implies the presence of a monomorphic antigen-presenting molecule that is an object of current research but remains incompletely defined. While selection on a monomorphic presenting molecule may seem unusual, similar mechanisms shape the alpha beta T cell repertoire including the extreme examples of NKT or mucosal-associated invariant T cells (MAIT) and the less dramatic amplification of public Vbeta chain rearrangements driven by individual MHC molecules and associated with resistance to viral pathogens. Selecting and amplifying public T cell receptors whether alpha beta or gamma delta, are important steps in developing an anticipatory TCR repertoire. Cell clones expressing public TCR can accelerate the kinetics of response to pathogens and impact host survival.

Keywords

gamma delta T cell receptor; TCR; Vgamma chains; public TCR; extrathymic selection

Discovering the gamma delta T cell receptor

Identifying rearranging genes and receptor diversity [1] was a sentinel achievement in the study of T cell biology. Complementing our knowledge of MHC restriction [2], the knowledge about T cell receptor (TCR) gene diversity allowed us to understand T cell

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ontogeny and how selection mechanisms guard against lethal autoimmunity. However, knowledge that other transcripts existed that were similar to but distinct from the beta chain or immunoglobulin genes [3] and discovery of the TCR gamma chain [4] alerted immunologists to be ready for some confusion about the nature of T cell antigen receptors and the control of repertoire diversity.

Early studies confirmed that TCR gamma chain was not required for recognizing MHC Class II determinants [5] or for cytotoxic T cell function [6]. We also learned that the gamma chain repertoire had limited diversity in mice [7] and was proposed initially to have a role mainly in thymic T cell development [8, 9]. Further studies showed that mixed lymphocyte responses of mouse T cells selected for a distinct subset of gamma chains [10] providing some support to the idea that a gamma chain-containing TCR was functional for antigen recognition. This was contradicted by early efforts to characterize the human gamma chain repertoire using cDNA cloning and DNA sequencing, which showed a low frequency of productive gamma chain transcripts in thymic or mature human T cells [11].

The characterization of a second TCR made from gamma and delta chains [10, 12–15] defined a unique lineage of T cells that were apparently independent of MHC restriction but required CD3 complex proteins for function and could have cytotoxic effector activity [16]. By showing that gamma delta TCR cells were the main lymphocyte type in epidermis or epithelia [17] it was possible to link TCR structure and anatomic distribution, a finding of fundamental significance for gamma delta T cell research. Within a brief but intense 4 years, the gamma chain was discovered and distinguished from alpha and beta chains, we learned that immune responses select for specific gamma chains, receptor type was linked to anatomic localization and a gamma delta TCR was functional, used CD3 complex proteins and defined an unique adult T cell lineage. These sentinel discoveries - defined important questions that only now (in 2015) are being solved and help us to understand fundamental aspects of TCR repertoire development and T cell function.

Structure of the genomic loci for gamma and delta chains

In order to define TCR repertoire diversity, several groups began to characterize the complexity of gamma delta T cell populations. Solving the structure of the gene locus and especially the 5 joining segments including the soon to be famous JP [18], were early contributions to understanding potential complexity of the expressed gamma chain. Numerous V gamma chain rearrangements were also described in neoplastic T cell lines [19, 20] or T cell clones [21]. Lefranc summarized the gamma locus structure in human beings by showing the presence of 2 constant regions genes, 5 joining segments and at least 14 variable gene segments [22]; these findings were supported by Font, et al. [23] and Quertermous, et al., the latter was reported with an alternate nomenclature for the variable and joining segments [24]. These early contributions defined the gamma chain locus (Figure 1) and provided important tools for understanding repertoire complexity and gamma delta T cell selection in human beings.

Far from being a rare or insignificant subset, gamma delta T cells are distributed throughout the body wherever alpha beta T cells can be found [25]. In blood, the repertoire is skewed

toward cells expressing a specific rearrangement of the Vgamma9 chain [26, 27], which is called Vgamma2 in an alternate nomenclature [24]. These common chains are made when Vgamma9 (Vgamma2) rearranges with the JP (Jgamma1 .2) joining segment, and splices to C1 to form the Vgamma9JPC1 (Vgamma2Jgamma1.2C1) chain [28]. The Vgamma9JP rearranged chain is paired mainly with Vdelta2 chains [26] even though there is no apparent restriction on pairing with other delta chains. While studies in mice characterized the broad utilization of all gamma chains and sequential delta chain expression during early mouse development (reviewed in [29, 30]), studies of gamma delta TCR in human peripheral blood leukocytes or leukocyte-derived neoplastic cells all pointed to overwhelming use of the Vgamma9 chain rearrangement paired with Vdelta2.

Skewing toward a single VJ rearrangement could be due to the orientation of individual Vgamma chains in relation to J segments [18, 28] or because antigen recognition properties of the Vgamma9-JP rearrangement lead to strong, positive selection in peripheral blood [26, 31]. A recent study of human gamma delta TCR repertoire at different stages of fetal development showed an early burst of Vgamma9Vdelta2 T cells with limited junctional diversity at around 23 weeks of gestation [32]. This population was dominant at 23 weeks but declined steadily until birth and was nearly gone in cord blood or neonatal venous blood [32]. Whether this early population of Vgamma9Vdelta2 T cells originated outside of the thymus, perhaps using a thymus-independent pathway similar to that described in fetal mouse [33, 34] or fetal human liver [33] is not known. Mouse fetal development is characterized by producing waves of gamma delta T cell populations that use different Vgamma and Vdelta chain combinations (reviewed in [29]). Early gamma delta T cell populations including cells arising in fetal liver, have limited junctional diversity due partly to low abundance of terminal nucleotidyl transferase (TdT) [35, 36], an enzyme required for adding non-templated nucleotides and creating junctional diversity during V-J recombination. Perhaps the early surge of Vgamma9Vdelta2 T cells during human fetal development reflects the contribution of fetal liver cells and limited junctional diversity in these cells [32] may be due to both preferential rearrangement and low TdT levels, although we cannot rule out the potential impact of positive selection by a TCR ligand present at specific times during fetal development.

If the abundance of individual Vgamma chains in neonates and adults was only controlled by sequential expression or preferred patterns of V-J rearrangements, we would expect to see the same distribution TCR in both thymocytes and circulating lymphocytes. In fact, the Vgamma9Vdelta2 TCR is rare in human thymocytes [37] even though there are no apparent restrictions on chain pairing [27, 38]. These and related findings steered us away from coordinated [39] or sequential [28] chain rearrangement as explanations for TCR abundance in the adult. In addition to overabundance of Vgamma9Vdelta2 chains, it was reported that Vdelta2 and Vdelta1 chain utilization vastly overshadowed any of the other Vdelta chains in peripheral blood lymphocytes [40] suggesting they contribute to TCR with higher function or promote recognition of abundant antigens. All of these observations pointed to positive selection based on TCR function as an explanation for the abundance of Vgamma9Vdelta2 TCR in human peripheral blood [41]. Ideas about functional selection were reinforced by several papers reporting specific accumulation of gamma delta T cells at sites of human infectious disease lesions [42], activation of gamma delta T cells in the primary immune

response to *Mycobacterium tuberculosis* [43, 44] or *M. leprae* [45], isolation of CD4-CD8-mycobacteria-reactive gamma delta T cell clones from rheumatoid arthritis synovial fluid or synovial membrane [46, 47] and both proliferative and cytotoxic effector responses to the Daudi B cell lymphoma line that might be due to expression of heat shock proteins in these cells [48, 49]. The Vgamma9Vdelta2 T cells were also found in demyelinating plaques from brains of patients with multiple sclerosis [50, 51], and epidermal lesions related to Oriental Cutaneous Leishmaniasis [52]. Clearly, the dominant circulating gamma delta TCR in adult humans beings is Vgamma9Vdelta2 and strong relationships with multiple types of disease imply this TCR is part of the immune response to common antigens.

Mechanisms shaping the adult circulating gamma delta TCR repertoire

Brenner's group [53] described the thymic and peripheral repertoire for gamma delta T cells and established more firmly the concept of extrathymic proliferation as a factor shaping our adult gamma delta TCR repertoire. They observed that Vgamma9Vdelta2 cells (the original paper used the Vgamma2Vdelta2 nomenclature) represented only a small fraction of total human thymocytes consistent with other reports [27, 54]. The Vdelta1 cells were abundant in thymus or blood at birth and remained at a fairly constant proportion of total CD3+ cells throughout life. The proportions of Vgamma9Vdelta2 T cells in thymocytes from post-natal thymi compared to fetal thymi were not different and the age-related changes occurred in the periphery of neonates or young children. Vgamma9Vdelta2 cells increased steadily in blood in terms of both absolute counts and proportion of CD3+ lymphocytes, until about 8 years of age. With advancing age, the proportion of CD45RO+ (memory marker) Vgamma9Vdelta2 cells also increased. These observations supported a view that increases in blood Vgamma9Vdelta2 T cells were due to extrathymic selection/expansion and that circulating cells were accumulating as antigen-experienced, memory cells [53]. In adults, the majority of circulating Vgamma9Vdelta2 T cells are CD45RO+ memory cells, compared to Vdelta1 cells that are mainly CD45RA+ naïve cells [55]. There were no correlations between MHC haplotype and patterns or rates of Vgamma9Vdelta2 T cell expansion; the constancy of Vdelta1 cells provided a good control for these studies [53]. The gamma delta TCR repertoire may vary with gestational age of the human fetus [37, 56] but the major changes were best characterized in neonates, children and adults. Later in adult life, complexity of the circulating Vgamma9 chain repertoire declines [57] possibly because of continuing positive selection and declining new cell synthesis. We know that positive selection is still active during adulthood because bone marrow transplant recipients eventually reconstitute the Vgamma9Vdelta2 TCR repertoire similar to healthy individuals [58, 59].

The processes of selection and extrathymic expansion are the major mechanisms responsible for deriving an adult gamma delta TCR repertoire from a rare fraction of thymocytes. When spectratyping was used to characterize the open reading frame length distribution for Vgamma9 chains in donors of different ages (measured with cDNA copied from T cell mRNA), the fetal repertoire (cord blood cells) displayed a bimodal distribution with a mode at 984 nucleotides and another at 993 nucleotides, while the adult repertoire displayed a skewed length distribution with a single mode at 993 nucleotides (numbered according to open reading frame length in nucleotides, Figure 2). This shift is due to the higher number of Vgamma9 chains that rearrange with the JP segment (most of which are between 990 and

996 nucleotide long) in adult peripheral blood compared to cord blood. This dramatic change has not been observed for alpha beta TCR populations and highlights the effect of selection pressures in shaping the adult gamma delta TCR repertoire. Within the population of 993 nucleotide-long Vgamma9 chains, up to 30% were reported to have the germline configuration for Vgamma9 and JP (detected in cDNA copies of mRNA) meaning there were no N nucleotide additions or deletions during recombination [60]. The potent selection for a single V-J rearrangement causes the adult repertoire length restriction [60]. In order to exert such strong selection pressure, a potent and nearly ubiquitous antigen, superantigen or self-antigen must be driving the process [61]. It was also reported that 98% of alpha beta T cells have rearranged Vgamma genes on both chromosomes but do not express the gamma delta TCR [62]. This finding implied that developing thymocytes first tried one or two different gamma chain rearrangements. If there was no signal for positive selection (because the rearrangements were non-productive), the beta chain rearranged on one or both chromosomes [63] and paired with the invariant chain to allow for positive selection and alpha beta T cell differentiation. This model implies that the rearranged gamma chain repertoire cannot be studied by sequencing genomic DNA from PBMC (as is done commonly to characterize the beta chain repertoire) because the non-productive sequences will exceed the productive sequences by at least 10-fold. Sequencing studies of the Vgamma9 chain repertoire have been used extensively to characterize age-related changes, disease impacts and responses to therapies; they depend on analyzing cDNA produced from T cell mRNA.

Stimulatory compounds and TCR structural requirements

Potent selection and amplification of gamma delta T cell suggests they have an important role in the immune response to infected or malignant cells or participate in regulation of immunity [64, 65]. Further, there must be a strong overlap between TCR ligands capable of driving extrathymic selection and ligands that are recognized on infected or transformed cells. By 1991 it was believed that extrathymic selection and amplification of Vgamma9Vdelta2 cells was driven by two types of TCR ligands: those found in tumor cell lines including Daudi lymphoma, Molt-4 lymphoma and MCF 7 breast cancer cells, and mycobacterial antigens [45, 66–69]. Deciding whether these antigens were alone responsible for extrathymic gamma delta T cell selection would require additional characterization of the repertoire and more precise information about the chemical nature of stimulatory ligands.

The subset of gamma delta T cells that responded to Daudi lymphoma cells or mycobacterial antigens, overlapped almost completely with the normal circulating T cell repertoire in blood [70]. The normal repertoire was characterized by a specific CDR3 motif formed when Vgamma9 rearranges with the JP segment [71–73]. Gene transfer studies showed that Vgamma9 rearranged with J2 or JP could pair with either Vdelta1 or Vdelta2 for responses to Staphylococcus enterotoxin A [74], arguing against pairing restrictions. However, the Vdelta2 gene segment was required for responses to prenyl pyrophosphates and swapping the Vdelta2 chain with a Vdelta1 chain abrogated reactivity [75]. There were substantial efforts to characterize Vdelta2 chain rearrangements and search for junctional elements that might be linked to extrathymic selection and functional responses [76]. The diversity of

Vdelta chain is high, partly due to presence of several D segments, and associations between sequence and function in delta chain are less clear than for the Vgamma9-JP rearrangement. Because the delta chain population is more complex and clear relationships with functionality are harder to discern, many investigators found that focusing on the gamma chain allowed studies of repertoire selection and immune responses that were easier to interpret.

Initial reports that organic compounds, including synthetic alkyl phosphates like monoethyl phosphate, were capable of stimulating Vgamma9Vdelta2 T cell proliferation [77] started the search for non-peptidic molecules that could explain why a single subset of T cells reacted uniformly to such diverse stimuli as tumor cells or mycobacteria. Subsequent studies expanded the list of potential stimulatory ligands and discovered a key and likely prominent role for pyrophosphate intermediates of mammalian sterol synthesis including isopentenylpyrophosphate (IPP) and its isomer dimethallylpyrophosphate [78]. Subsequently, IPP has been used to stimulate expansion and effector function of Vgamma9Vdelta2 T cells in many laboratories. The Vgamma9 chain repertoire of IPP-expanded cells is very similar to the repertoire of circulating PBMγ delta T cells [79] indicating a close relationship between IPP responses in vitro and extrathymic selection/amplification mechanisms shaping the natural repertoire. Since IPP is a ubiquitous molecule made in all mammalian cells, it may be one of the missing links between extrathymic selection and TCR ligands that trigger functional responses. Structural studies identified features of the gamma delta TCR [80, 81] that are needed for responses to IPP or related compounds (collectively designated as phosphoantigens).

Based on the assumption (not supported by biophysical evidence) that phosphoantigens would bind the Vgamma9Vdelta2 TCR directly, a positively charged pocket at the cleft between two CDR3 loops was identified as the putative phosphoantigen recognition site [82]. Further, most phosphoantigen-reactive clones have a conserved hydrophobic residue, encoded by N nucleotides, at position 97 in the CDR3 region of Vdelta2 (Leu, Ile, Val and Gly being predominant) suggesting both chains are contacting stimulatory prenyl pyrophosphates [71]. Gene transfer and mutagenesis studies tested these hypotheses and confirmed that phosphoantigen responsiveness depends on: a) JP-encoded lysine residues, particularly at position 108 in the CDR3gamma region [83, 84]; b) an N-encoded hydrophobic residue in position 97 near the Vdelta2-Jdelta junction [84]; arginine 51 in CDR2delta [84]. A more recent mutational study described the roles for CDRgamma and CRDdelta in antigen recognition, with most of the critical residues being germ-line encoded [85].

These findings also helped to identify other stimulatory compounds for gamma delta T cells. The non-mevalonate pathway for isoprenoid synthesis in some bacteria or protists produces (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) that is a stimulator of Vgamma9Vdelta2 T cells [86]. This molecule is similar to IPP but much more potent and is produced by organisms using the LytB pathway [87]. In mammalian cells, the same pathways that produce phosphoantigens are also targeted in the treatment of osteonecrosis/osteoporosis. Aminobisphosphonates are inhibitors of the enzyme farnesyl pyrophosphate synthase that catalyzes sequential condensation of IPP and dimethallylpyrophosphate to

form geranylpyrophosphate and farnesylpyrophosphate. By blocking the farnesyl pyrophosphate synthase condensation reaction, IPP is expected to accumulate and reach stimulatory levels. Indeed, commonly used aminobisphosphate drugs stimulate Vgamma9Vdelta2 T cell proliferation in vitro and render tumor cells or monocytes more susceptible to cytotoxic killing [88]. Aminobisphosphonate stimulation requires antigen-presenting cells (APC) that may be tumor cells, macrophages or dendritic cells. Mouse cells do not function as APC for human Vgamma9Vdelta2 T cells. This is likely due to the fact that murine cells do not express BTN3A1, a molecule that is conserved in a variety of placental mammals which also express Vgamma9 and Vdelta2 genes, suggesting a co-evolution of TCR and BTN3A1 [89]. In the last few years, genomic, functional and structural evidence pointed to an essential role for butyrophilin3A1 (BTN3A1) in phosphoantigen-mediated activation, either as an intracellular sensor [90–92] or as a presenting molecule for prenyl-pyrophosphates [93]. BTN3A1 most likely interacts with additional molecules in accessory cells in order to elicit Vgamma9Vdelta2 T cell responses [94, 95] but it alone is insufficient for phosphoantigen responses and must be supplemented by other, yet unidentified molecules encoded on human chromosome 6 [94]. The role of BTN3A1 has been discussed extensively in recent reviews [96, 97] including a contribution from Erin Adams in this issue. The evolving story of BTN3A1 drives home the uncomfortable but interesting idea that stimulation and effector function of Vgamma9Vdelta2 T cells may require a ligand, which has not been identified. While BTN3A1 certainly contributes to Vgamma9Vdelta2 T cell activation by phosphoantigens, the molecules used to distinguish infected or malignant cells from their normal counterparts remain undefined and could hold the key to a better understanding of repertoire selection and the functional utility of these cells.

Knowing that rhesus or baboon APC support the response of human Vgamma9Vdelta2 T cells to HMBPP [91] but other species cannot provide antigen presenting cells, we wondered whether differences among germline Vgamma9 chains might be related to phosphoantigen recognition. Considering the structural requirements for TCR antigen recognition [83, 84] and the species restriction - [98], we would expect to find differences in key Vgamma9JP residues among primate species that would match the patterns for functional recognition. When nucleotide (Table 1A) or amino acid (Table 1B) Vgamma9 and JP sequences were aligned for human beings, chimpanzees, rhesus or cynomolgus macaques, night monkeys (related to squirrel monkeys) and common marmosets, a clear pattern emerged. The C terminus of Vgamma9 chain is highly conserved among humans, chimps and Old World macaques but diverges in the New World night monkeys and marmosets. Within the JP segment, Old World macaques differ from both human beings and chimpanzees near the amino terminus, but the required Lysine at position 108 (residue 6 in our table) is conserved and the lysine in position 107 (residue 5) is substituted by another positively charged amino acid (arginine). The hydrophobic amino acids at positions 109 (7) and 111 (9) are also conserved. Since all of these species react to phosphoantigen stimulation, species-specific variation may not define the antigen recognition pocket but rather the site for binding a monomorphic antigen-presenting molecule that varies sufficiently among species to select for differences in the Vgamma9 and JP germline coding regions.

These studies defined the range of natural compounds capable of stimulating Vgamma9Vdelta2 T cells, provided insight into TCR structure and brought us closer to understanding species specificity for antigen presentation. Importantly, stimulatory Aminobisphosphonates include clinically approved drugs that might be used to activate Vgamma9Vdelta2 T cells in patients. The list of diseases that could benefit from Vgamma9Vdelta2 T cell activation includes HIV, where the greatest depletion of these cells was observed (reviewed recently [99]), mycobacterial diseases, malaria, cancer and others. This is an active field of research and beyond the scope of this review, but current and future clinical studies depend ultimately on our understanding of the Vgamma9Vdelta2 TCR repertoire, how to control expansion or function of these cells, and how to exploit repertoire analysis to monitor the impact of clinical therapeutic trials.

An adult Vgamma9 repertoire dominated by public clonotypes

The mature, adult Vgamma9Vdelta2 TCR repertoire is created by a process of extrathymic selection and the majority of these cells both use the JP joining segment and respond to phosphoantigens or γ aminobisphosphonate stimulation. Despite using a common V-J rearrangement, circulating Vgamma9Vdelta2 cells are still diverse due to N region variation, which is caused by deletions and random additions of non-template nucleotides occurring during V-J recombination. The vast majority of cells expressing the Vgamma9-JP rearrangement will respond to phosphoantigen [79] however, the magnitude of response in terms of proliferation, varies depending on the gamma chain sequence. Within a population of Vgamma9Vdelta2 T cells from healthy adults, each individual clone may proliferate faster or slower after phosphoantigen stimulation and the relative rates for individual Vgamma9 chains are preserved among unrelated donors, indicating it is a property of the TCR [100]. Despite these differences, the repertoire is relatively stable over at least 20 years, showing retention of at least 85% of circulating gamma chain sequences over this time period [100]. Because the circulating repertoire is overwhelmed with phosphoantigen-reactive cells and they are mostly CD45RO memory cells, there is a rapid and uniform response to stimulation that seems more like an innate immune response than an acquired T cell response. Indeed, more than 1 in 40 human circulating memory T cells is a Vgamma9Vdelta2 cell capable of responding to phosphoantigen and this constitutes the largest memory response in man.

What are the critical features of a gamma delta TCR repertoire? Of course, repertoire selection depends heavily on the nature of restricting elements. The alpha beta TCR depends on polymorphic major histocompatibility molecules while the Vgamma9Vdelta2 TCR recognizes what seems to be a monomorphic presenting molecule that remains incompletely defined. We know the presenting molecule for Vgamma9Vdelta2 TCR is monomorphic because of the very high degree of overlap in the repertoire of unrelated, healthy adults. The canonical or germline gamma chain sequences predominate in most, but not all individuals. However, the germline amino acid sequence (clonotype) can be produced by non-germline nucleotide sequences and the high representation of this clonotype irrespective of how it was produced, shows that selection and expansion depends on functional properties of the TCR. When identical clonotypes or nucleotide sequences (nucleotypes) of Vgamma9 chains are found in more than one unrelated donor, they are designated as public sequences. This

differs from the convention for alpha beta T cells where public refers to common use of a single V-J rearrangement and does not require nucleotide or amino acid identity. In a small survey of healthy donors, 51 public Vgamma9 chain clonotypes were identified from a sample of African American and Caucasian American donors [101]. More than 70% of African American public clonotypes were present in Caucasian Americans, even though baseline levels of circulating Vgamma9Vdelta2 T cells were 2–4 fold higher in the Caucasian American group [101]. Further, the rank order of public clonotypes in terms of abundance was similar between both groups and could be used to outline the characteristics of a “healthy” repertoire. The “healthy” repertoire could in turn be distinguished from a disease repertoire that was impacted by HIV, even when the Vgamma9Vdelta2 T cell population was reconstituted after prolonged antiretroviral therapy [102]. The studies on HIV disease (reviewed in [99]) also highlighted the value for having a T cell population with redundant responses to a single phosphoantigen. When damaged by a disease that reduces the cell population and repertoire complexity, a redundant repertoire continues to have functional responses and can be reconstituted by expansion and selection of the residual cells in addition to newly generated cells at any time in life. The rarity of individual alpha beta T cells makes it unlikely that an individual TCR would ever be produced twice in the same person and any damage due to disease that extinguishes an alpha beta T cell clone would require replacement of that clone or using another with similar recognition properties. The disadvantage of redundancy is that Vgamma9Vdelta2 T cells can recognize fewer individual antigens, even though they are involved in a great number of immune responses with their “less diverse” TCR repertoire.

Advantages of public clonotypes for T cell immunity

Despite the differences between MHC-restricted and MHC-unrestricted T cells, some features of the alpha beta TCR repertoire are strikingly similar to the gamma delta TCR examples (Figure 3). Polymorphism, when used to describe antigen-presenting molecules, is a term referring to a population of individuals. Each individual within the population has only one set of MHC genes. Thus, each adult only faces polymorphism in case of blood or tissue transplantation and reproduction; for most of life, including thymocyte selection that creates the mature T cell repertoire, population complexity of the histocompatibility locus is not very important. We also know that strong selection of public Vbeta chains amplifies particular Vbeta rearrangements and depends on a particular MHC allele. Despite the potential for very large TCR diversity, the CD8 T cell response to Epstein-Barr virus (EBV), cytomegalovirus (CMV), influenza virus and other human viral diseases is surprisingly limited among individuals with the same MHC haplotypes. This was first noted because epitope-specific T cell clones from unrelated donors shared the same patterns of alloreactivity [103, 104] and more detailed analyses showed a strong conservation of common Vbeta-J rearrangements despite N region diversity. Among individuals with the same MHC haplotype, selection for public clonotypes precedes a first encounter with foreign antigen and must reflect the influence of TCR binding to MHC during thymic selection; this mechanism leads to amplification of public alpha beta TCR clonotypes [105, 106]. If an individual has the right MHC to select for public clonotypes capable of recognizing important pathogens, they may generate more rapid, protective T cell responses

upon natural exposure and have a better chance for survival. The chances for selecting public clonotypes is influenced by convergent recombination, a process that provides multiple pathways for generating the canonical or germline TCR chain sequences [107–109], that was also noted in the studies on TCR reconstitution after HIV infection and prolonged therapy [102]. The more extreme examples of public alpha beta TCR include invariant NKT and mucosa-associated invariant cells (MAIT) that depend on public TCR chains [110]. NKT and MAIT, similar to Vgamma9Vdelta2 T cells, recognize non-peptidic antigens and appear to be selected by monomorphic antigen presenting molecules. Selection and amplification of public clonotypes is an important and common process for shaping TCR repertoire.

We generally speak about the alpha beta TCR repertoire as being a random sampling of the potential genetic diversity using all Vbeta and Jbeta segments plus N region variation, coupled with similar variation in the alpha chain. Calculators produce astronomical numbers for the potential diversity of alpha beta T cells. However, the adult TCR repertoire actually looks far from random in most studies, and the repertoire is imprinted by selective amplification that is biased toward recognition of common antigens and/or antigen presenting molecules. Strong anticipatory selection, meaning selection prior to pathogen exposure, shapes each repertoire and impacts the subsequent kinetics of response when individual T cell clones are called into action during infection or malignancy. If the pre-existing TCR repertoire has a higher proportion of cells capable of responding to a single antigen, then the kinetics of response are faster and there is a greater chance for survival. Selective amplification is a basic aspect of immune memory, but selection of public clonotypes prior to pathogen exposure is less appreciated as a determinant of successful immunity. Whether common MHC molecules expand cell clones expressing public alpha beta TCR or phosphoantigens are skewing the gamma delta T cell repertoire, a highly selected, anticipatory repertoire dominated by public TCR chains can be key to effective cellular immunity. When we argue whether gamma delta T cells belong to innate or adaptive immunity, or compromise by proposing they are a “missing link” between innate and adaptive responses, we are really talking about a highly focused memory T cell subset that dominates the circulating gamma delta T cell population and responds to a single stimulus with the kinetics of an innate immune cell. For public alpha beta T cell clones, the kinetics of response are probably similar to Vgamma9Vdelta2 T cells but at a smaller scale since public alpha beta T clones are still rare. These circulating, memory T cells bearing public T cell receptors are capable of rapid responses to foreign threats and provide a crucial survival advantage for the host.

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Highlights

- We review discovery of the gamma delta T cell receptor locus.
- We describe extrathymic selection and shaping of the adult TCR repertoire.
- Extrathymic selection impacts the function of gamma delta T cells.
- Selection for public gamma delta or public alpha beta are compared.
- We review the impact of selecting public TCR on T cell function.

Recombination and mRNA Splicing Generate the Vgamma9-JP-C1 T cell Receptor Chain

Figure 1. Structure of the human gamma chain locus and steps in assembly of a Vgamma9JPC1 chain

The figure depicts a portion of human chromosome 7 (p15 to p14) and is not drawn to scale. Showing in the germline are 14 Vgamma chain genes or pseudogenes, 5 J segments and 2 constant regions. The filled purple, green yellow or red boxes depict functional sequences. Filled gray boxes are pseudogenes. White boxes are open reading frames not shown to be functional. N region indicates the site for Nontemplated nucleotide addition and identifies junctional diversity introduced during V-J recombination. Sequences lacking the N region are considered germline. Adapted from the International ImMunoGeneTics information system® viewable at: <http://atlasgeneticsoncology.org/Genes/TRGID268.html>

Figure 2. Vgamma9 chain length distributions in cord blood (N=54) or adult peripheral blood (N=46) specimens

This is a spectratype analysis that measures the length of coding region sequences in expressed, Vgamma9 chain mRNA. Individual columns heights indicate the proportion of all coding regions of a specific length. Coding region length varies due to the individual J segment used for each rearrangement and the extent of N region additions or deletions. Neonatal Vgamma9 chains (cord blood) display a bimodal distribution with modes at 984 and 993 nucleotides of length (open columns). Adult Vgamma9 chains (peripheral blood) display a skewed, unimodal distribution with a mode at 993 nucleotides of length (filled columns). The adult Vgamma9 repertoire is a higher frequency of chains between 990 and 996 nucleotides length (in particular the 993 nucleotide component) compared to the cord blood specimens (*** $p < 0.0001$). Skewing of the adult repertoire reflects the increased proportion of cells bearing JP compared to the neonatal repertoire.

Comparing Thymic and Adult T cell receptor repertoire for alpha beta or gamma delta T cells

Figure 3. A model showing important steps causing the adult Vgamma9 TCR and alpha beta TCR repertoires to be altered by positive selection and extrathymic expansion

Above the dark bar we depict events of thymic T cell differentiation. The double negative precursor cells assort into gamma delta or alpha beta T cells depending on productive TCR chain rearrangements and thymic selection mechanisms. This produces heterogeneous cell populations where the potential expanded Vgamma9JPVdelta2 T cells or alpha beta T cells are only a fraction of the total. Below the dark line we depict the consequences of extrathymic selection and expansion of individual clones that shape the adult repertoire. In the case of cells expressing the Vgamma9JP rearrangement and pairing with Vdelta2, phosphoantigen recognition is a common feature. The presence of ubiquitous phosphoantigens drives selective amplification resulting in a skewed adult population that is dominated by the Vgamma9JP+ cells (red centers). In the case of alpha beta T cells, individual T cell clones (yellow centers) may be expanded due to the presence of favorable major histocompatibility restricting elements. This selection process also results in repertoire skewing, though to a lesser extent than was seen for gamma delta T cells.

Table 1

A. Nucleotide sequence differences in germline encoded V γ 9 C-terminus and JP N-terminus among six primate species.				
		Nucleotypes		
Organism (common name)	Origin	V γ 9	JP	
<i>Homo sapiens</i> (Human)		GCC TTG TGG GAG GTG	CAA GAG TTG GGC AAA AAA ATC AAG GTA TTT GGT CCC GGA ACA AAG CTT ATC ATT ACA G	
<i>Pan troglodytes</i> (Chimpanzee)	Great Ape	--- --- --- --- ---	--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -	
<i>Macaca mulatta</i> (Rhesus macaque)	Old World	--- --- --- --- ---	--- C-- --T --- -G- --- G-- --- C-- --- --- --- --- --- --- --- --C --- --- -	
<i>Macaca fascicularis</i> (Cynomolgus macaque)	Old World	--- --- --- --- ---	--- C-- --T --- -G- --- G-- --- C-- --- --- --- --- --- --- --- --C --- --- -	
<i>Aotus nancymae</i> (Ma's night monkey)	New World	--- --C --- --- A--	--- --- --T --- --- --- --- --- --G --- --- --- --- --- --- --- --C --- --- -	
<i>Callitrix jacchus</i> (Common marmoset)	New World	--- --C --- --- -C-	--- --- --T --- --G --- G-- --- --G --- --- --- --- --- --- --- --C --- G-- --- -	

B. Amino acid sequence differences in germline encoded V γ 9 C-terminus and JP N-terminus among six primate species.				
		Clonotypes		
Organism (common name)	Origin	V γ 9	JP	
<i>Homo sapiens</i> (Human)		A L W E V	Q E L G K K I K V F G P G T K L I I T	
<i>Pan troglodytes</i> (Chimpanzee)	Great Ape	- - - - -	- - - - - - - - - - - - - - - - -	
<i>Macaca mulatta</i> (Rhesus macaque)	Old World	- - - - -	- Q F - R - V - L - - - - - - - - -	
<i>Macaca fascicularis</i> (Cynomolgus macaque)	Old World	- - - - -	- Q F - R - V - L - - - - - - - - -	
<i>Aotus nancymae</i> (Ma's night monkey)	New World	- F - - M	- - F - - - - - - - - - - - - - -	
<i>Callitrix jacchus</i> (Common marmoset)	New World	- F - - A	- - F - - - V - - - - - - - - - V -	