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RESEARCH HIGHLIGHT

Clonal expansion shapes the human Vδ1T cell receptor repertoire

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The γδ cells are a unique population of T lymphocytes that combine innate-like features and adaptive-type responses and play an important role in the early host response to infections and malignancies. Different from αβ T cells, γδ T cells recognize a limited set of antigens, which are shared by a variety of microbial pathogens and tumor cells in a non-MHC restricted manner;[1] thus, these cells use the TCR in a manner similar to a pattern recognition receptor (PRR). Moreover, whereas αβ T cells require antigen- and cytokine-driven clonal expansion, γδ T cells are equipped with immediate effector functions.[1] However, the potential γδ repertoire with junctional diversity is estimated at ~10^{18}, which is much greater than the αβ repertoire (~10^{16}), thus raising questions concerning the forces governing the selection of such a huge TCR repertoire during ontogeny and whether and how the γδ TCR repertoire is shaped under physiological and pathological conditions.

In a recent issue of *Nature Communication*, Davey *et al.*[2] used amplicon rescued multiplex (ARM)-PCR and next-generation sequencing to investigate the clonal selection of a γδ TCR repertoire that was negative for the Vδ2 chain in healthy adults and in cord blood. As expected, in all individuals, the $Vδ2^-$ γδ T cell population was dominated by $Vδ1^+$ T cells paired with diverse Vγ chains and displaying a mixed terminally differentiated effector memory ($CD27^-$ $CD45RA^+$) or naive ($CD27^+$ $CD45RA^+$) phenotype.[3] Strikingly, in most (70%) adult individuals, remarkably strong focusing (i.e., a reduction in diversity) of the $Vδ1^+$ TCR repertoire toward a small number of individual clonotypes ($\leqslant 10$) was observed, and this effect was evident for both TCRγ and TCRδ chains. In contrast to this focused adult subgroup, markedly less focused repertoires were observed in seven individuals (30%); notably, this minority of individuals, defined as "diverse adult donors", were primarily cytomegalovirus (CMV) seronegative and included the youngest members of the cohort, suggesting an age-dependent modification of the $Vδ1^+$ TCR repertoire.

To investigate how the $Vδ1^+$ TCR repertoire differed in early life, Davey *et al.*[2] conducted comparable TCR repertoire analyses on the $Vδ1^+$ population obtained from cord blood. $Vδ1^+$ cells dominate the cord blood γδ repertoire and express Vδ1 paired with diverse Vγ regions. The cord blood TCRδ1 and TCRγ CDR3 sequences were extremely unfocused, in contrast to focused the adult Vδ1 repertoires but similar to the unfocused adult samples, and comprised numerous low-frequency clonotypes, the most prevalent of which represented $<1.30\%$ and $<2.17\%$ of the total unique CDR3s detected for TCRγ and TCRδ, respectively (Figure 1). As expected, detailed comparisons of the CDR3 length within and between individuals indicated that in $Vδ1^+$ T cells, the mean CDR3δ1 length was substantially greater than that of CDR3γ (mean 54 versus 33 nucleotides); strikingly, comparisons of the ten most prevalent TCRγ and TCRδ1 clonotypes (typically accounting for $>50\%$ of the repertoire) from each donor revealed private sequences (e.g., present only in one individual but absent in any other individuals either at a nucleotide or amino acid level). Therefore, the Vδ1 TCR repertoire was overwhelmingly private, with different TCR clonotypes present in each individual (Figure 1), and comparison of Vδ1 repertoire data with age- and sex-matched TCRβ repertoire data revealed that Vδ1, as a repertoire, was even more private than TCRβ. Moreover, the most frequent clonotypes were detected in subsequent ARM analyzes conducted 12–18 months later, and in most donors, the hierarchy of prevalent clonotypes was broadly conserved in

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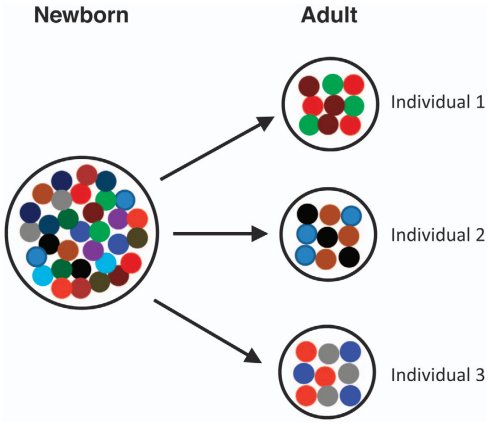

Figure 1 The neonatal (cord blood) TCR Vδ1 repertoire is extremely unfocused and private. In most adult individuals, the Vδ1$^+$ TCR repertoire is strongly focused, with up to 50% of the repertoire comprising the 10 most abundant TCRγ and TCRδ1 clonotypes. Moreover, the Vδ1 TCR repertoire was overwhelmingly private, with different TCR clonotypes present in each individual. The nature of the forces governing the peripheral clonal selection of the human Vδ1 TCR repertoire and the role of CMV remains to be determined.

both analyses, which clearly indicated that the clonotypic expansions prevalent in the Vδ1T cell repertoire are stably maintained over time.

Thus, these findings indicate that Vδ1T cells undergo profound expansions of TCR clonotypes in the periphery from an initially completely unfocused and private repertoire, which is highly suggestive of an adaptive-type immune response.

Hence, selection of the Vδ1 TCR repertoire differs in several key aspects from that of Vγ9Vδ2T cells, which are the major γδ population in peripheral blood and secondary lymphoid organs. In contrast to the Vδ1 population, the Vγ9Vδ2 TCR repertoire data from Davey and colleagues confirmed the highly restricted CDR3 lengths, including prevalent Vγ9-JP sequences of limited complexity that were common to multiple individuals, with CDR3γ9 lengths of 11–18 amino acids, and >50% of the CDR3γ9 contained a typical 14 amino acid sequence in all donors. Moreover, analysis of the 10 most frequent TCRγ and TCRδ clonotypes in Vδ2$^+$ cells from each donor revealed that the CDR3γ9 sequences were public and constrained in length.[4] Although CDR3δ2 sequences were relatively private compared with TCRγ9, more CDR3δ2 sequences than CDR3δ1 sequences were shared between donors. Therefore, in stark contrast to Vδ1$^+$ T cells but analogous to iNKT and MAIT populations, the Vγ9Vδ2 population expresses a semi-invariant TCR.

Consistent with these data, Dimova *et al.*[5] detected prevalent Vγ9 sequences that were present at birth in multiple individuals, regardless of pathogen exposure. These observations are consistent with a semi-invariant, innate-like biology for the Vγ9Vδ2 subset that is compatible with the polyclonal activation of these cells via phosphoantigens.

Importantly, Vδ1 clonal expansion was concomitant with phenotypic differentiation involving the loss of secondary lymphoid homing markers and the upregulation of effector molecules. The vast majority of clonal populations occurred in an effector memory/terminally differentiated CD27$^{lo/neg}$ CD45RA$^+$ population, whereas clonotypes showing naive-type CD27^{hi} CD45RA$^+$ expression were diverse, suggesting that Vδ1 TCR repertoire focusing was accompanied by a transition from a naive to an effector phenotype.

Davey and colleagues used other surface markers to better define the cell differentiation state, and thus Vδ1 CD27^{hi} cells express other naive molecules, such as IL-7Rα, CD28, CCR7, and CD62L, which were absent in the CD27$^{lo/neg}$ compartment. Functional analysis showed that the naive CD27^{hi} subset proliferated in response to IL-7 stimulation, while the CD27$^{lo/neg}$ required IL-15 for proliferation, and this latter is a typical feature of terminally differentiated effector memory γδ T cell subsets,[6] and both populations proliferated in response to anti-CD3/CD28 and anti-TCR γδ mAb. Moreover, CD27$^{lo/neg}$ cells contained granzyme A, B and perforin in contrast to CD27^{hi} cells and expressed CX3CR1. The overall analysis showed that Vδ1 clonal expansion was accompanied by a phenotypic and functional transition: of note, cord blood and adult Vδ1T cells with an unfocused TCR repertoire had a naive phenotype and did not express cytotoxic molecules, whereas clonally expanded Vδ1 populations were preferentially effector cells equipped with cytotoxic activity.

Collectively, these findings reveal a fundamentally adaptive behavior for Vδ1T cells, which is likely governed by the TCR, and strongly support a model involving the clonal selection of naive Vδ1T cells expressing TCRs enabling responses to yet-unknown antigens, accompanied by differentiation to a terminally differentiated effector memory phenotype. These authors argue against the idea that Vδ1 clonotypic focusing merely reflects an immunological imprint of past challenges and instead are more suggestive of a long-lived, highly specific, functional γδ T-cell memory that enables augmented responses to recurrent challenges, akin to classical immunological memory, although importantly, not MHC restricted.

This model shares several key tenets with classical adaptive immunity but differs critically by being MHC-unrestricted, and it represents an unconventional mode of adaptive immune surveillance. Thus, this study presents many questions. Although the identity of the forces underlying Vδ1 TCR repertoire focusing is unknown, it might include microbial antigens and/or self-molecules.[7] Furthermore, although the private TCR repertoires may reflect responses restricted to each individual, they do not formally exclude the likelihood of the degenerate recognition of conserved ligands by diverse TCRs.

CMV infection, which has been strongly associated with Vδ2$^-$ T-cell responses,[8,9] is not relevant to the data

of Davey and colleagues, since CMV-seronegative individuals also exhibited extreme clonotypic focusing. Additionally, it is important to determine why ~30% of the adults in the study of Davey and colleagues retain a largely unfocused, naive Vδ1 repertoire.

Moreover, the study of Davey and colleagues exclusively analyzed $V\delta1^+$ T cells in the peripheral blood. No evidence on tissue-resident $V\delta1^+$ T cells has been reported, although these cells represent a majority of the γδ T cell population.[10] This finding is important because residence in a non-lymphoid tissue, regardless of whether the tissue is normal or has undergone tumor transformation, serves as a major determinant of the phenotypic and functional characteristics of tissue-resident Vδ1 and Vδ2T cells (Meraviglia *et al.*, unpublished results). Because relatively little is known about human tissue-resident γδ T cells, sequencing the TCR repertoire of cells isolated from several tissues is therefore necessary to better increase the current knowledge of the selection of the γδ TCR repertoire and to determine the nature and mode of antigen recognition and the role of γδ T cells in tissues. These aspects may be translationally relevant and may provide novel therapeutic avenues in anti-tumor immune responses.[11–13]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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