

## RESEARCH HIGHLIGHT

# Human $\gamma\delta$ T cells: rapid, stable and clonally reactive

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Gamma delta ( $\gamma\delta$ ) T cells are a unique and conserved population of lymphocytes. They are considered ‘unconventional’ T cells because they represent a relatively small subset of T cells and are defined by the expression of heterodimeric T-cell receptors (TCRs) that are composed of  $\gamma$  and  $\delta$  chains. Data show that  $\gamma\delta$  T cells function in many types of immune responses and immunopathologies. They provide protective immune responses against a wide range of pathogens and directly eliminate infected or stressed cells.<sup>1,2</sup> Gamma delta T cells produce various cytokines and chemokines that have the capacity to modulate other immune and non-immune cells.<sup>3</sup> They can also facilitate the maturation of dendritic cells and modulate the activation of conventional  $\alpha\beta$  T cells.<sup>4,5</sup> Although  $\gamma\delta$  T cells can be protective, they can also be deleterious. For instance, the potent cytotoxicity of  $\gamma\delta$  T cells and their ability to produce IFN- $\gamma$  are considered protective against cancer, whereas IL-17-producing  $\gamma\delta$  T cells are associated with tumors.<sup>6</sup> Nevertheless, the combination of conventional adaptive functions that are inherent in their TCRs, as well as their

rapid innate-like responses and potential to modulate initial immune responses, makes  $\gamma\delta$  T cells attractive candidates for immunotherapy.<sup>7</sup>

In contrast to conventional T cells that express  $\alpha\beta$  TCR and whose development and function are well characterized, the human  $\gamma\delta$  T-cell lineage remains enigmatic. This is attributed to their poorly defined antigens, the unconventional mechanisms by which they respond to immunological stimuli as well as the difficulty to track them *in vivo*. Unlike  $\alpha\beta$  TCRs, the repertoires of human and murine  $\gamma\delta$  TCRs were thought to be narrow, individual and stable. It is currently unclear if a narrow TCR repertoire is genetically imprinted or whether it is shaped postnatally. Furthermore, it is uncertain whether human  $\gamma\delta$  T cells undergo clonal proliferation in response to microbial challenge and if they can provide adaptive immunity.

In a recent issue of *Nature Immunology*, Ravens *et al.*<sup>8</sup> studied human  $\gamma\delta$  T cells and provided new insight regarding two fundamental questions in  $\gamma\delta$  T-cell immunobiology. First, how are  $\gamma\delta$  TCR repertoires shaped in humans? Second, do human  $\gamma\delta$  T cells undergo clonal proliferation upon activation with their cognate antigen? To address these questions, the authors used RNA-based next-generation sequencing (NGS) to comprehensively analyze the repertoires of both TCR $\gamma$  and TCR $\delta$  chains in  $\gamma\delta$  T cells purified from patients undergoing transplantation of hematopoietic stem cells. A prospective longitudinal cohort study was performed, and based on

previous data, ~20–30% of the patients were expected to experience reactivation of cytomegalovirus (CMV). This scenario enabled the authors to monitor the  $\gamma\delta$  T-cell pool before and after transplantation and allowed them to determine how human  $\gamma\delta$  T cells are shaped during ontogeny. Moreover, by tracking  $\gamma\delta$  TCR repertoires before and after CMV reactivation, the authors were able to examine how  $\gamma\delta$  T cells respond to a major microbial challenge.

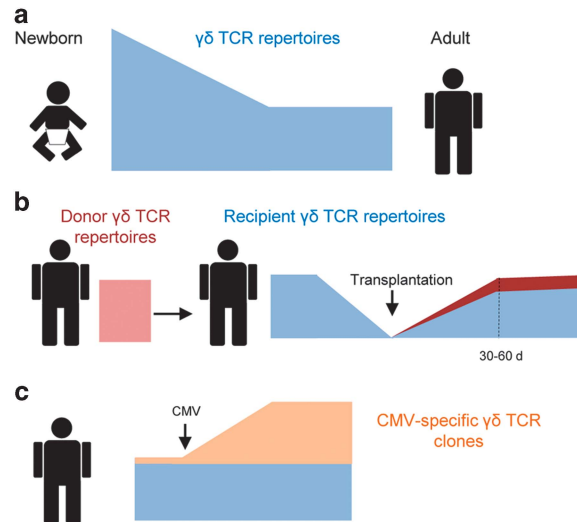
In the first part of the study, the repertoires of steady-state rearranged TCR $\gamma$  and TCR $\delta$  genes (*TRG* and *TRD*, respectively) were assessed in patients before transplantation as well as in healthy adults (the control group). Gamma delta T cells purified from cord-blood samples were also analyzed and represent the TCR repertoires at birth. The authors revealed that, at birth, the repertoires of *TRG* and *TRD* are highly diverse. Nevertheless, during life, the TCR diversity was reduced due to preferential proliferation of certain  $\gamma\delta$  T-cell clones (Figure 1a). These adult  $\gamma\delta$  TCR repertoires were found to be remarkably stable, as they showed no significant alteration over 90 days. It should be noted that this study was the first to perform a NGS analysis of human *TRD* repertoires, which revealed that the *TRD* repertoire was consistently more diverse than the *TRG* repertoire at birth as well as in adults. Furthermore, by comparing the sequence overlap on individual  $\gamma\delta$  TCR repertoires, the authors found that the human *TRG* repertoire contained public clones (that is, they

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**Figure 1** Dynamics of  $\gamma\delta$  T-cell receptor (TCR) repertoires. (a) After birth, the TCR repertoires of  $\gamma\delta$  T cells present high diversity. This changes during life as the  $\gamma\delta$  TCR repertoires become more focused and narrow, likely due to environmental and genetic factors. The adult  $\gamma\delta$  TCR repertoires remain remarkably stable in healthy adults. (b) In individuals undergoing transplantation of hematopoietic stem cells,  $\gamma\delta$  T cells are rapidly reconstituted in the host and present  $\gamma\delta$  TCR repertoires typical to adults. This newly generated repertoire differs greatly from the  $\gamma\delta$  TCR repertoires of both the donor and recipient before the transplantation, suggesting that  $\gamma\delta$  T cells differentiate *de novo* in the recipient's thymus. Nevertheless, donor-derived  $\gamma\delta$  T cells also contribute directly to the reconstituted  $\gamma\delta$  T-cell pools, probably via homeostatic proliferation. (c) Cytomegalovirus (CMV) infection or reactivation represents an intense challenge to the immune system. CMV-reactive  $\gamma\delta$  T-cell clones exist in the  $\gamma\delta$  TCR repertoires of stem cell recipients and, upon activation, massively proliferate in an antigen-specific manner, leading to a significant increase in frequency among total  $\gamma\delta$  TCR repertoires.

were present in many people), whereas the TRD repertoire appeared to be unique to individuals and thus was considered private.

Next, the impact of hematopoietic stem cell transplantation on  $\gamma\delta$  TCR repertoires was examined. The authors discovered that  $\gamma\delta$  T cells were reconstituted rapidly within 30–60 days and that their repertoires remained stable over 180 days. Although the reconstituted repertoires resembled adult TRG and TRD repertoires, they were fundamentally different from the original repertoires of the patients prior to transplantation. Moreover, the reconstituted  $\gamma\delta$  TCR repertoires also differed from the donor repertoires (Figure 1b). The authors concluded that  $\gamma\delta$  T cells were generated *de novo* in the adult thymus of the transplanted patients. Nevertheless, it was also evident that some of the donor  $\gamma\delta$  T clones contributed directly to the recipient  $\gamma\delta$  T cells, most likely via homeostatic proliferation.

In the last part of the study, the authors focused on patients experiencing CMV reactivation after transplantation. Upon initial reconstitution of new  $\gamma\delta$  TCR repertoires, reactivation induces

major perturbations in the already rearranged TRG and TRD repertoires. The authors identified individual  $\gamma\delta$  T-cell clones that massively proliferated after the reactivation (Figure 1c). A single-cell analysis of the TRG and TRD sequences further confirmed that the proliferating  $\gamma\delta$  T-cell clones are indeed CMV-reactive.

While the work by Ravens *et al.* addresses fundamental issues in  $\gamma\delta$  T-cell immunobiology, it also highlights several questions that require further examination. First,  $\gamma\delta$  T cells are located in peripheral tissues, in which they play a critical immunological role. The present work examined  $\gamma\delta$  T cells in the blood; it is still unclear how reconstitution after transplantation affects the  $\gamma\delta$  TCR repertoires of tissue-resident  $\gamma\delta$  T cells. Second, a large portion of  $\gamma\delta$  T cells develop during embryonic development; these cells are likely to encounter a unique signaling environment during their differentiation that might not exist in the adult thymus after transplantation. Thus, although the  $\gamma\delta$  TCR repertoires of reconstituted  $\gamma\delta$  T cells resemble 'normal' adult repertoires, it is uncertain whether these cells will function as

embryonic  $\gamma\delta$  T cells, migrate to the peripheral tissues and have the capacity to regulate local immunity. Third, with regard to the robust expansion of virus-specific  $\gamma\delta$  T-cell clones, further investigation is required to dissect their immunological functions, including their ability to protect the host and become memory cells similar to conventional  $\alpha\beta$  T cells.

In conclusion, the study by Ravens *et al.* provides compelling evidence demonstrating that (i)  $\gamma\delta$  TCR repertoires become oligoclonal during adulthood but remain highly diverse and stable; (ii) upon transplantation of hematopoietic stem cells,  $\gamma\delta$  T cells reconstitute rapidly, and their  $\gamma\delta$  TCR repertoires retain the overall complexity and proportion typical of adult  $\gamma\delta$  TCR repertoires; and (iii) CMV reactivation induces robust proliferation of CMV-reactive  $\gamma\delta$  T-cell clones, suggesting that  $\gamma\delta$  T cells might be capable of generating anti-viral adaptive immune responses. These findings should have direct clinical implications, since the rapidly differentiating  $\gamma\delta$  T cells are considered to have beneficial properties after transplantation. Thus, this work has

important translational and prognostic implications; in the future,  $\gamma\delta$  T cells might be considered during hematopoietic stem cell transplantation.

### CONFLICT OF INTEREST

The author declares no conflict of interest.

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