JEM Journal of Experimental Medicine

INSIGHTS

γδ T cells: The first line of defense for neonates

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A distinct CD83-expressing subset of γδ T cells are enriched in preterm infants with sepsis, providing insights into their functional maturation dynamics in settings of homeostasis and disease (León-Lara et al. https://doi.org/10.1084/jem.20231987).

The neonatal period is one of extreme vulnerability, when newborns emerge from the protective maternal environment and must establish their own immunity to multiple environmental and pathogenic insults. The adaptive immune system, mediated by antigen-specific T cells, takes years to fully develop (Connors et al., 2023; Rudd, 2020), and the specific immune mechanisms that can protect the newborn during the first weeks of life remain incompletely understood. γδ T cells are a unique T cell subset that develop early in ontogeny during fetal life. They express a T cell receptor (TCR) comprising a γ and δ chain and can respond to non-peptide antigens through their TCR independent of MHC, and can also initiate "innate-like" responses through cytokine-mediated signaling. In humans, γδ T cells develop within the fetal thymus, and consist primarily of Vδ2-expressing cells paired with the Vγ9 TCR and exhibit effector functions including production of pro-inflammatory cytokines and cytolytic mediators (Perriman et al., 2023; Qu et al., 2022). By contrast, Vδ1 cells, which can pair with a variety of γ chains, are less prevalent in the thymus in early life, but exhibit more adaptive-like features where they can clonally expand to diverse pathogens and come to dominate in tissues over the course of life (Davey et al., 2018). Despite this knowledge, the dynamics of γδ T cells in early life and their role in protection from infection has proven difficult to characterize in humans.

In this issue of *JEM*, León-Lara et al. (2024) investigate the role and function of human γδ T cells in sepsis from the blood of a novel longitudinal cohort of preterm infants. Neonatal sepsis is a life-threatening condition caused by dissemination of bacterial pathogens into the circulation. Preterm infants are particularly susceptible due to their increased need for invasive medical procedures that disrupt mucous membranes. Additionally, the lack of a mature adaptive immune system in early life infers alternative cellular mechanisms are required for rapid protection when newborns are exposed to a myriad of new antigens.

The authors examined the maturation dynamics and functional responsiveness of γδ T cells by sampling blood from infants with sepsis and healthy controls at four timepoints: 0–14 days (sepsis period), 21–35 days, 6–10 mo, and 13–19 mo (sepsis-free periods). They conducted high-dimensional flow cytometry, TCR sequencing (TCR-seq), and single-cell transcriptome profiling by single-cell RNA sequencing (scRNA-seq) to investigate post-thymic γδ T cell maturation at homeostasis, during sepsis, and after resolution. They found that Vγ9$^+$ γδ T cells—commonly described as innate-like and most likely paired with the Vδ2 chain—were specifically enriched in preterm infants that had sepsis, whereas Vδ1 cells were not. Following resolution of sepsis, there were no differences in contribution of either subset to the T cell pool between sepsis donors and healthy controls. The authors then sought to identify the source of Vγ9$^+$ cell enrichment in sepsis and whether they were fetally derived and polyclonal or were generated through extrathymic selection of post-natal clones indicated by clonally expanded populations. Following TCRδ-seq, infants with sepsis were enriched for a cluster of TCRs consisting of *TRDV2* and *TRDJ3*, specifically during the sepsis period compared to after the resolution of sepsis, as well as healthy controls. These TCRs had very few N-additions and were highly diverse, indicating they were fetally derived (Manchorova et al., 2022). Their enrichment during active sepsis infection provides evidence that Vδ2Vγ9 cells may respond to systemic infection in the first days of life in the absence of a fully mature adaptive immune system.

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The authors then conducted scRNA-seq with paired scTCR-seq to comprehensively assess the underlying dynamics of γδ T cell subset maturation at homeostasis and

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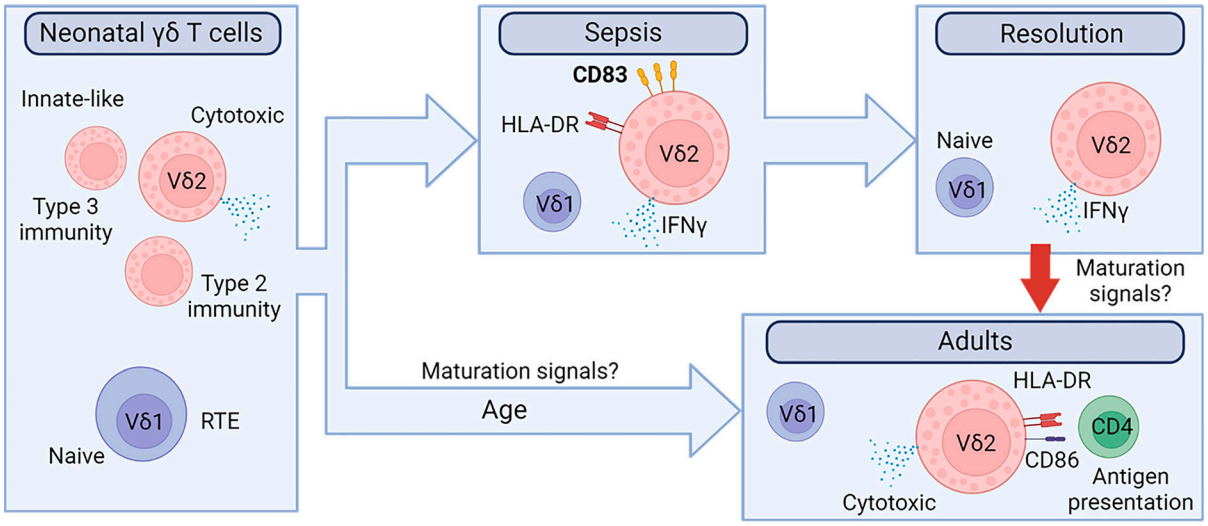

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The dynamics of γδ T cell subsets across infection and age. Vδ1 cells exhibit signatures of naive T cells or recent thymic emigrants (RTE) in neonatal blood while Vδ2 cells are heterogeneous with cytotoxic, type 1, type 2, and type 3 immune profiles. During neonatal sepsis, Vδ2 cells transiently upregulate CD83 while IFNγ expression is maintained after resolution. Adult Vδ2 γδ T cells acquire the ability to stimulate and present antigen to CD4 T cells through upregulation of CD86 and various HLA Class II molecules.

during sepsis. Notably, this study included a pair of twins, one with sepsis and one without, to control for genetic factors associated with highly variable transcriptional profiles observed in humans. They identified a number of subsets across the sepsis and sepsis-free time periods, including naive cells, cells with type 1, type 2, and type 3 immune signatures, and cytotoxic cells, all have which have previously been described in studies of cord blood and human thymic γδ T cells (Perriman et al., 2023; Tan et al., 2021). Additionally, they describe a previously uncharacterized HLA-DR^{hi} subset expressing CD83 transcripts associated with activation and antigen processing (León-Lara et al., 2024), while they lacked expression of other effector genes. Over the course of the sampling time period, Vδ1 cells expressed signatures of recent thymic emigrants, whereas Vδ2Vγ9 cells were predominantly cytolytic, indicating distinct dynamics of thymic egress. Importantly, the dynamics of subset frequencies in infants with sepsis was distinct; they possessed lower frequencies of cytotoxic Vδ2Vγ9 cells throughout the course of sepsis compared to healthy controls, along with an enrichment of CD83-expressing Vδ2Vγ9 T cells specifically during the sepsis period, whereas cells with an IFNγ signature persisted in enhanced frequencies compared to healthy controls (see figure).

Next, the authors compared the upregulation of specific activation markers between infants and adults after stimulation to assess whether CD83 upregulation was specific to neonatal γδ T cells. When stimulated with (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate and IL-2, neonatal Vγ9Vδ2 cells upregulated CD83, whereas adults preferentially upregulated CD86, consistent with their known capabilities of antigen processing and CD4 T cell stimulation (Tyler et al., 2017). The CD83^{+} subset from neonates with sepsis also exhibited high expression of various HLA molecules; therefore, the authors asked whether this subset served a specialized antigen presenting function during disease (see figure). Perhaps surprisingly, only adult Vδ2Vγ9 cells were capable of processing antigen and inducing CD4 T cell stimulation, indicating the CD83^{+} subset in neonates with sepsis serves an alternative function (see figure). These experiments indicate that not only are γδ T cells differentially affected by an infection during infancy, but their resultant responses change across life, highlighting the need to consider age when investigating γδ T cells for therapeutic manipulation.

CD83 is a member of the immunoglobulin superfamily and can be expressed on mature dendritic cells, activated T cells, and B cells in order to regulate over-exuberant inflammatory responses (Grosche et al., 2020). A possible function for CD83 on γδ T cells could be the modulation of immune responses to attenuate the inflammatory environment during sepsis. CD83-stimulated monocytes have been shown to suppress T cell proliferation as well as secretion of IL-2 and IFNγ (Chen et al., 2011); therefore, CD83^{+} γδ T cells could similarly suppress inflammation through interactions with activated monocytes. Additionally, CD83 is highly expressed on regulatory T cells where it is essential for tolerance and modulating secretion of inflammatory cytokines (Doebbeler et al., 2018). This potentially indicates a dual role for Vδ2Vγ9 cells specifically in neonatal sepsis, whereby CD83^{-} cells are capable of cytotoxic responses to the pathogenic insult, and the CD83^{+} subset serves as a regulatory population to dampen the inflammatory response. Further investigation is required to elucidate the roles of CD83 on γδ T cells during infancy and sepsis, and the mechanisms by which it functions.

The authors present a comprehensive analysis of γδ T cell maturation at homeostasis and during sepsis. This knowledge builds on our current understanding of the subset composition, functional capabilities

and development of γδ T cells in the fetal thymus (Parker and Ciofani, 2020; Tieppo et al., 2020), and bridges the gap between these studies and the extensively characterized Vδ2 subsets in adult blood. The longitudinal sampling of neonatal blood during and after systemic disease is difficult to accomplish—particularly for infants and children. Their study therefore provides valuable new insights into how γδ T cells respond during severe infection and after resolution prior to development of adaptive immunity. It will be important to determine the involvement of CD83⁺ expressing γδ cells to other types of infections (e.g., viruses) and the mechanisms by which CD83 is specifically upregulated in infant γδ T cells compared to adults. This study also highlights the disparity in cellular responses of a neonatal immune system compared to the fully mature system in adults, emphasizing the importance of studying the developmental dynamics of immune cells at specific life stages, in settings of both homeostasis and disease.

The characterization of γδ T cell development has improved dramatically over the past few years in that we now have extensive knowledge of their development in the fetal thymus, fetal and infant blood, and adult blood, particularly for Vγ9Vδ2 cells, which predominate in circulation. However, γδ T cells, particularly the subset expressing the Vδ1 TCR, are the original "tissue-resident" T cell, where studies in mice have demonstrated they can seed peripheral tissues in early life and conduct effector functions in situ (Jin et al., 2010). A recent study in mice has implemented multimodal profiling of γδ T cells across various tissue sites (du Halgouet et al., 2024), and a new study characterized human γδ T cells in different tissues and over age (Gray et al., 2024). It will be important to integrate data from human clinical studies as done here by León-Lara et al. (2024) with tissue profiles to understand the biology and role of γδ T cells in host defense at different life stages.

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