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"Microbiota regulates γδ intraepithelial lymphocyte homeostasis and enhances activity against microbial invasion"

by
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Wednesday, May 5, 2021
11:00 A.M.
WebEx:
https://rutgers.webex.com/rutgers/j.php?MTID=mcdb1008505c2727a9191a5451685fdcd

ABSTRACT

Intraepithelial lymphocytes expressing the γδ T cell receptor (γδ IEL) provide first line of defense to limit intestinal injury and infection. Although the presence of an intact microbiome is not required for γδ IEL development, the loss of gut microbes after birth reduces the number of IELs. How the microbiota influences this sentinel population is poorly understood. Based on the known role for commensal-induced tonic type I IFN signaling in the lamina propria lymphocyte homeostasis, we hypothesized that microbiota-induced IFNα/β receptor (IFNAR) signaling contributes to the maintenance of the γδ IEL compartment. First, morphometric analysis was performed on the jejunum of GFP γδ T cell reporter mice (TcrdEGFP, WT) and TcrdEGFP IFNAR KO mice. It revealed a 2-fold increase in the number of GFP^+ γδ IELs in IFNAR KO mice compared to WT housed in a standard barrier facility (SBF). Further, 5-Ethynyl-2′-deoxyuridine (EdU) was administered to assess γδ IEL proliferation *in vivo*. Consistent with the increased number of γδ IELs, SBF IFNAR KO γδ IELs exhibited a 50% increase in proliferation relative to SBF WT. Bulk TCR sequencing and clonotype analysis of γδ IELs isolated from SBF WT and IFNAR KO mice showed polyclonal TCR γδ repertoires, indicating that the expanded γδ IEL population was not due to clonal expansion. Surprisingly, the relative proportion of γδ IELs was skewed toward $Vγ7^-$ subsets in SBF IFNAR KO mice. EdU incorporation within each Vγ subset revealed that SBF IFNAR KO mice exhibit increased proliferation in both $Vγ7^-$ and $Vγ7^+$ IEL populations relative to SBF WT. Whereas very few γδ IELs populate the SBF WT gut at one week after birth, SBF IFNAR KO γδ IELs were increased 11-fold at this early timepoint.

Interestingly, IFNAR KO mice rederived into a cleaner, enhanced barrier facility (EBF) had a normal γδ IEL compartment resembling that of WT mice, indicating that the γδ IEL hyperproliferative phenotype occurs independently of IFNAR signaling. The transfer of dirty bedding from SBF IFNAR KO mice to EBF WT or IFNAR KO breeding cages was sufficient to induce the γδ IEL hyperproliferative phenotype in both the breeders and their offspring, and broad-spectrum antibiotic treatment prevented this transmission. Separately-housed SBF WT and IFNAR KO mice were crossed to generate F2 littermates, which all exhibited the γδ IEL hyperproliferative phenotype regardless of genotype. Evidence of horizontal and vertical transmission of the γδ IEL hyperproliferative phenotype led us to analyze the fecal microbiota. The fecal microbiota of WT and IFNAR KO mice housed in both facilities was analyzed by 16s rRNA sequencing. Greater microbial diversity was observed in mice with the γδ IEL hyperproliferative phenotype relative to those that did not. Moreover, 6 amplicon sequence variants (ASV) were strongly associated with the γδ IEL hyperproliferative phenotype.

Intravital imaging analysis demonstrated that SBF F2 WT γδ IELs migrate twice as frequently into the lateral intercellular space (LIS) and at higher rates of speed compared to SBF WT. Consistent with this enhanced surveillance behavior, SBF F2 WT mice exhibit decreased bacterial load in the spleen and liver respectively compare to SBF WT mice after oral infection with *Salmonella* Typhimurium.

Overall, we have serendipitously discovered a novel γδ IEL hyperproliferative phenotype that arises early in life and is dependent on the intestinal microbiota. SBF IFNAR KO fecal microbiota is both necessary and sufficient to promote the expansion of the γδ IEL compartment. This hyperproliferative and hypermotile γδ IEL phenotype provides protection against systemic dissemination of *Salmonella* infection. These findings may provide the foundation to develop microbiome-based approach to enhance γδ IEL immunosurveillance with the goal to reduce microbial translocation and help maintain mucosal homeostasis.