Microbiota-associated cytokines robustly enhance the ability of FoxP3+ Tregs to suppress EAE

Zhou and Cobb

Julie Y. Zhou and Brian A. Cobb

Department of Pathology, Case Western Reserve University, Cleveland, OH

The prevalence of multiple sclerosis (MS) in the United States increased by 33% between 2001 and 2014, and is among many inflammatory and autoimmune disorders to see such rises. A potential contributor to this trend was posited 3 decades ago by the Hygiene Hypothesis, which speculated that immune dysregulation result from a shift in microbial exposure in both the exogenous environment and the endogenous microbiome. Here we identify and adopt a strategy used by a common commensal gut microbe, Bacteroides fragilis, to suppress excessive immune activity. Bacteroides fragilis produces a capsule containing polysaccharide A (PSA), which has been shown to suppress a host of inflammatory diseases in an IL-10-dependent manner. Despite being a polysaccharide, we discovered that PSA is endocytosed, processed, and presented by antigen presenting cells through MHCII and αβTCR engagement just like conventional protein antigens, and clonally expands CD4+FoxP3-CD25-CD45Rblo cells of an effector memory subset (RbloTEM). RbloTEM from both PSA experienced and inexperienced mice suppress peripheral inflammation in an IL-10 dependent way, demonstrating the intrinsically suppressive nature of this T cell subset. Interestingly, RbloTEM do not produce the IL-10 necessary for suppression of disease. Instead, RbloTEM produce both IL-2 and IL-4, which signal to FoxP3+ regulatory T cells (Tregs) to produce robust amounts of IL-10. Using novel FoxP3RFP/IL-10GFP dual reporter mice, we demonstrate here that the combination of IL-2 and IL-4 (IL-2/IL-4) induces synergistic IL-10 expression specifically in Tregs, enhances Treg proliferation while preferentially favoring the expansion of IL-10+ Tregs, and dramatically increases the suppressive ability of Tregs. Through promoting a robust and rapid Treg response, we show that IL-2/IL-4 works in preventative, concomitant, and therapeutic delivery schedules to suppress experimental autoimmune encephalomyelitis (EAE), including its associated clinical score, demyelination, MOG-specific conventional T cells, and production of the pro-inflammatory IL-17A. All in vitro experiments were run in technical and biological triplicates and confirmed in independent repeats. EAE trials were run using female mice (n=10 for MOG conditions, n=4 for vehicle conditions). These data suggest that IL-2/IL-4 can be further studied to reduce or stop MS progression.