The Role of and G-protein γ subunit 2 in the Pathogenicity of $CD4^+$ T cell in Experimental Autoimmune Uveitis

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Autoimmune uveitis, an autoimmune non-infectious inflammatory eye condition, is studied using experimental autoimmune uveitis (EAU) *in vivo*. EAU is driven by T cells, demonstrated by the induction EAU in naïve mice via the transfer of retina antigen specific pathogenic $CD4^+$ T cells (PTCs). The purpose of this study is to understand the mechanism underlying PTCs pathogenicity in EAU. We performed RNA sequencing analysis on C57BL/6 mice comparing PTCs and endogenous $CD4^+$ T cells (ETCs) and found 135 differentially expressed genes ($Padj<0.05$). Of these, 106 genes were found to have human orthologues and from those genes, 22 gene candidates that were highly associated with human autoimmune diseases were identified. Three genes of interest are: CD226, linked to multiple sclerosis, type 1 diabetes, rheumatoid arthritis, and juvenile idiopathic arthritis; Klrk1/NKG2D, associated with Behçet's disease; and G-protein γ subunit 2 (Gng2), correlated with multiple sclerosis.

Published single-cell RNA sequencing studies of spontaneous uveitis in AIRE-/- knockout mice show that Gng2 expression peaks in Th1 cells, followed by Th17, T regulatory cells, and finally, Th2 CD4+ T cell subtypes. Using quantitative real-time PCR, we found that in OTII transgenic mouse $CD4^+$ T cells and splenocytes, Gng2 mRNA expression increases after stimulation with anti-CD3/28 and is upregulated in retinas during early EAU. Similarly, in human T cell leukaemia cell lines (Jurkat and CCRF-CEM cells) GNG2 mRNA levels were enhanced after TCR stimulation. Using siGNG2 RNAi knockdown and the $G\beta_1\gamma_2$ small molecule inhibitor gallein, we observed that inhibiting Gng2 impedes chemotaxis toward CXCL12 in Jurkat and CCRF-CEM cells, while activation induced upregulation of Gng2 enhances chemotaxis toward CXCL12. Likewise, gallein also inhibited $CD4^+$ T cell chemotaxis towards CXCL12. Gallein also slows proliferation by inhibiting G2/M cell cycle entry in Jurkat and CCRF-CEM cells. In conclusion, targeting Gng2 in $CD4^+$ T cells could be a potential therapeutic strategy to treat autoimmune uveitis.