OCULAR IMMUNITY
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W.19. Cytokine Profile of Splenocytes During Experimental Autoimmune Uveoretinitis (EAU)

Darren Lee, Andrew Taylor
Harvard Medical School, Boston, MA

After the resolution of experimental autoimmune uveitis (EAU) TGF-β producing Treg cells emerge in the spleen, but not in EAU Melanocortin 5 Receptor KO (MC5r(-/-)) mice. We induced EAU in C57BL/6 mice to determine when the TGF-β producing T cells emerge in the spleen. Secreted TGF-β, IFN-γ, and IL-17 concentrations were measured from activated splenocytes collected throughout EAU. We found splenocytes from wild type EAU mice produced IFN-γ and IL-17 throughout the course of active disease when stimulated with ocular autoantigen, IRBP. TGF-β production gradually increased through disease progression and was maximum at resolution. IFN-γ and IL-17 production declined with the resolution of EAU. In contrast, MC5r(-/-) mice produced higher concentrations of IFN-γ and lower concentrations of TGF-β. Our results suggest that there is an appearance of IRBP-specific Th1 and Th17 cells in the spleen of mice just before maximum uveoretinitis and a low level of IRBP-specific TGF-β producing T cells. The TGF-β producing T cells become the dominant response as the disease resolves. In the course of EAU there is parallel change in the autoantigen-response in the spleen. IRBP-specific TGF-β producing T cells emerge as the disease progresses and become dominant as uveitis resolves, which is dependent on MC5r expression.

W.20. Alternative Activation of Macrophages by Immunomodulating Neuropeptides from Retinal Pigmented Epithelial Cells

Andrew Taylor, Norikuni Kawanaka
Harvard Medical School, Boston, MA

We have found that conditioned media of mouse retinal pigmented epithelial cells (RPE CM) in a posterior eyecup induced alternatively activated macrophages (MØ) through the neuropeptides α-melanocyte stimulating hormone (α-MSH) and neuropeptide Y (NPY). Immunostaining showed that all the $CD64^+$ $F4/80^+$ $CD11b^+$ cells in the healthy retina were simultaneously $Arginase1^+$ and $NOS2^+$; whereas, in wounded retinas the cells were either $Arginase1^+$ or $NOS2^+$, but not both. When we treated primary MØ with NPY NOS2 expression was induced, and when treated with α-MSH Arginase1 expression was enhanced. Only when treated with the combination of α-MSH and NPY did we see coexpression of $Arginase1^+$ and $NOS2^+$ in the MØ. When the MØ were treated with RPE CM from laser-burned eyes, POMC-knocked mice, or with α-MSH or NPY absorbed RPE CM two different stained populations of MØ ($Arginase1^+$ or $NOS2^+$) were seen. In addition, the MØ went into apoptosis when treated with α-MSH-absorbed CM, laser-burn RPE-CM, or POMC(-/-) mice RPE-CM. Our results suggest that RPE mediate through α-MSH and NPY the induction and selection of alternatively activated MØ as part of ocular immune privilege. Loss of this regulation could make the eye susceptible to autoimmune disease or unregulated wound repair. Supported by EY010752.

W.21. Retinal Laser Burn Abrogates Ocular Immune Privilege

Kenyatta Lucas, Joan Stein-Streilein
Schepens Eye Research Institute, Boston, MA

It is known that immune privilege allows for immune protection in the eye in the absence of inflammation. Recently we reported that retinal laser burn (RLB) abolished ocular immune privilege in both the burned and the contralateral (non-burned) eye. Antigen inoculation into the anterior chamber failed to induce anterior chamber associated immune deviation (ACAID) in both the eye that received the RLB and the contralateral eye as early as 6hrs and as late as 21 days post RLB treatment. Additionally, aqueous humor from the burned and the contralateral eyes was unable to convert antigen presenting cells (APC) to tolerogenic APC (tolAPC). Here we show, RTPCR analysis of mRNA extracted from eyes of RLB or non-treated mice showed little or no change in TGFβ, but the inflammatory cytokine, IL-6, was selectively up regulated in the burned eye mRNA samples of mice post RLB and not the contralateral eye. However, the genetic absence of IL-6 (IL-6 KO mice) did not restore the ability to induce ACAID post-RLB. In addition, the immunosuppressive function of retinal pigment epithelial cells was compromised in eyecups post RLB. Data shows fewer T cells increase their Foxp3 expression after coculture with eyecups harvested from mice post RLB compared to non-treated eyecups. These data suggest that RLB alters the immunosuppressive environment of the eye by not only altering the immunosuppressive/ inflammatory cytokine ratio but also by interfering with the immunosuppressive function of pigment epithelial cells that protect the borders of the eye.