examined retina structure and cellular composition in the developing PD-1-/- retina. Here we report no gross structural retina defects in the absence of PD-1. However, we observe a very significant, yet transient, increase in both total number of Brn3a+ RGCs and proportion of Brn3a+ RGCs within the RGC layer, coinciding with the peak of naturally occurring PCD at postnatal days P2 to P4. Furthermore, the downstream intracellular phosphatase Shp-2 is activated during this time, and in the absence of PD-1, Shp-2 activation is dramatically dysregulated. Based on these findings, we predict that PD-1 absence will cause functional defects in light-evoked retina electrical activity, within both the developing and mature retina. Our studies establish the importance of PD-1 signaling in developmental neuronal culling and present a novel mechanism for neuronal loss during retinal and cerebral inflammation, where PCD is triggered by the interaction of infiltrating inflammatory cell PD-ligands with neuronal PD-1.

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Su.55. Immune Surveillance of Privileged Tissue is Increased by Non-specific Stimuli

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Experimental autoimmune uveoretinitis (EAU) is an animal model for human inflammatory eye disease. EAU is induced in B10.RIII mice by immunisation with the interphotoreceptor retinoid binding protein (IRBP) peptide 161-180 peptide and adjuvant. This generates antigen specific $CD4^+$ T cells which infiltrate the eye leading to a peak of disease approximately 2 weeks after immunisation. Normal eyes are almost devoid of $CD4^+$ cells, which allows us to visualise this infiltration at high resolution, by collagenase digestion of inflamed retinal tissue and the quantification of leucocyte cell numbers by flow cytometry. Using this approach, we found that $CD4^+$ and $CD11b^+$ cell numbers were increased at d5 post immunisation when compared to unimmunised animals. This effect was not antigen specific, because it could also be observed in mice immunised with ovalbumin peptide 323-339 in adjuvant or when the mice received pertussis toxin alone. However, the cellular infiltrate caused by immunisation with OVA 323-339 was transitory and leucocyte numbers return to normal levels by d13. In mice immunised with IRBP 161-180, the leucocytosis persists, and increases dramatically between d10 and 13, corresponding to the peak of clinical disease. To investigate whether the d5 infiltrate was adjuvant specific, mice were infected with PR8 influenza virus and eyes analysed for cellular infiltrate. We found an increase in retinal $CD11b^+$ cell numbers at d5 post infection with influenza virus. The data demonstrate that a number different non-specific stimuli can increase the leukocyte content of an immune privileged organ, and therefore the level of immune surveillance.

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Su.56. Development of Panuveitis and Cataract in a Patient with X-linked Agammaglobulinemia

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Introduction: Although panuveitis and/or cataract are very rarely reported among hypogammaglobulinemic patients; association of panuveitis/cataract with X-linked agammaglobulinemia (XLA or Bruton's disease) is not known. Here, we report an XLA patient who developed panuveitis and cataract over time. Case presentation: A 19 year-old boy has been suffering from recurrent pneumonia and arthritis since childhood. At 3 years of age, he was diagnosed with bronchiectasis confirmed by CT, and underwent left lower lobectomy when he was 9 years old. 1 year later, XLA was suspected and diagnosis was established by typical clinical and laboratory findings and by mutation analysis of the Btk gene. As IVIG therapy once a month was initialized, frequency of infections significantly decreased. About 3-4 years later, he began to experience blurred vision and redness of both eyes. Eye examination was consistent with bilateral panuveitis. He also developed cataract in his right eye, may have occurred as a complication of panuveitis. He had a cataract surgery following the long-term treatment of uveitis for about 2 years. Currently, through ongoing ophthalmic corticosteroid therapy, his ocular problems are nearly in control with rare exacerbations. Conclusion: This case suggests that panuveitis and cataract might be rare manifestations of XLA. And it looks like IVIG supplementation may not prevent the development of these ocular manifestations throughout disease.

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Su.57. Autoimmune Inflammatory Eye Disease is Associated with the TNF-α dependant Generation of Myeloid Cells in the Target Organ that Suppress Auto-reactive $CD4^+$ T Cell Proliferation

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Experimental autoimmune uveoretinitis (EAU) is an *in vivo* model of human posterior ocular inflammation and can be induced in susceptible mouse strains by immunization with ocular proteins or peptides. EAU requires the generation of antigen-specific $CD4^+$ T cells, whose stimulation within the eye leads to macrophage activation and structural damage. This macrophage activation is TNF-α dependent and we have previously shown that $TNFR1^{-/-}$ mice are resistant to EAU induction. Here we investigated the phenotype and function of infiltrating leukocytes during EAU in C57/BL6, $TNFR1^{-/-}$ and B10.RIII mice which had been immunized with uveitogenic peptides from interphotoreceptor retinoid binding protein emulsified in adjuvant. Infiltrating leukocytes were released by enzymic digestion, but despite containing high numbers of $CD4^+$ T cells, they did not proliferate in response to

restimulation with either the immunizing peptide or plate-bound anti-CD3/CD28, although nitric oxide was generated. When $CD4^+$ T cells were separated from whole eye digests, they could proliferate in response to anti-CD3/CD28. In addition, the purified $CD4^-$ cell fraction was able to suppress T cell proliferation in *in vitro* cultures, when added at ratios as low as 1:25. This $CD4^-$ infiltrate contained high numbers of $CD11b^+Gr\text{-}1^+$ myeloid cells and the $CD4^+$ T cells had low expression of CD3ξ. In contrast, single cell suspensions from whole eye digests of immunized $TNFR1^{-/-}$ animals did proliferate *in vitro*. We suggest that the generation of these myeloid suppressor cells is an important regulatory component of a sustained autoimmune inflammation, and that the function of these cells is controlled via TNFR1.

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Su.58. Inflammatory Response and Involvement of Immune Components in a Laser Induced CNV (Choroid neovascularization) Mouse Model

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We used a laser induced CNV mouse model to investigate the nature and kinetics of the involvement of the immune response. Three additive and complimentary cellular and immunological techniques including 1) immunohistochemistry staining. 2) flow cytometry analysis and 3) antibody-based depleting or blocking strategies were used for functional analyses. Flat-mount tissue preparations enables quantitatively and qualitatively measuring CNV lesions while vibrotome tissue preparations is best for visualizing the infiltration of inflammatory cells. Flow cytometry is suitable for qualitatively and quantitatively assessing the total number of infiltrating immune cells during CNV development. There is an early influx of neutrophils, macrophages, NK cells and microglia cells within 72 hours. Early CNV lesions (within 72 hrs) are marked by edema and a random infiltration of immune cells, while lesions at a later stage (7 days) are characterized by a much reduced edema but very organized infiltrating membrane along the region of laser disruption. Microglial cells are also among the earliest to arrive to the laser lesion and persist for as long as 2 weeks. While no significant differences were observed in comparing wild type mice to SCID, or Rag-1 or CD25+ Treg cells depleted mice, there was a significant increase of CNV lesion when an LFA-1 antibody was administered. In conclusion, we have successfully applied several strategies to qualitatively and quantitatively analyze the kinetic involvement of immune cells during a typical laser induced CNV. Our data revealed a complicated but a very well orchestrated infiltration of immune components, mostly innate immune cells, during CNV. It appears that LFA-1 may play a beneficial role for the reversal of CNV. Our data suggest a pivotal role of immune response during CNV development.

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Su.59. Thrombospondin Facilitates Generation of Regulatory Cells Induced by TGFβ-treated Antigen Presenting Cells

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Our laboratory has determined key molecular mechanisms utilized by the TGFβ-exposed APCs from the eye that induce a form of peripheral tolerance and associated regulatory cells. In this regard, recently Thrombospondin (TSP1), a matricellular matrix protein capable of activating latent TGFβ, was revealed to play a critical role in the tolerance induced by TGFβ-exposed APCs. We now examine its role in generating regulatory T cells and show that TGFβ-treated APCs derived from $TSP1^{-/-}$ mice fail to generate regulatory cells in vitro . In contrast to typical regulatory cells induced by TGFβ-exposed wild-type APCs, effectors activated by similarly treated $TSP1^{-/-}$ APCs do not show reduced proliferation as detected by CFSE-dilution or secretion of IFNγ and IL-4 as measured by ELISA. Also, these effectors fail to secrete increased TGFβ like normal regulatory T cells. Furthermore, these effectors revealed a Th17 phenotype with increased IL-17 and IL-6 secretion detectable by ELISA upon their re-stimulation with anti-CD3. Although such a phenotype of T cells can be attributed to the inability of APCs to activate latent TGFβ in the absence of TSP1, interestingly we noted that such inhibition of IL-17 was possible using a TSP-derived peptide that does not contain TGFβ activating portion of TSP. These results identify an ability of TSP1 to directly regulate the phenotype of activated T cells that is independent of its ability to activate latent TGFβ. Collectively, these data reveal how TSP1 expressed by TGFβ-exposed APCs facilitates the generation of regulatory cells involved in peripheral tolerance.

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Immuno-oncology

Su.60. A Novel Vaccine Concept for Treatment of Cervical Cancer

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Therapeutic vaccines based on well-defined tumor associated antigens (TAAs) or synthetic peptides derived from such antigens represent an attractive approach because of practicality and applicability to broad tumor types. However, efforts to develop therapeutic vaccines have been unsuccessful so far primarily due to possible evasion mechanisms employed by progressing tumors and the weak nature of TAAs. Adjuvants with potent activity specific for immune cells may remedy this problem. Inasmuch as adaptive immune responses are critical to immune surveillance and the eradication of existing tumors, and such responses are regulated by CD28 and TNFR costimulatory molecules, we herein tested whether adjuvants based on costimulatory molecule (chimeric SA-4-1BBL) as an adjuvant, has therapeutic efficacy. The choice