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DETECTION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN EXPERIMENTAL AUTOIMMUNE UVEORETINITIS (EAU) CORRESPONDS WITH ENHANCED EXPRESSION OF INTERFERON-γ.

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Purpose: We have suggested a role for nitric oxide as a mediator of the autoimmune destruction of the retina in our rat model of EAU. In previous immunohistochemical studies we detected the iNOS enzyme only during and at the site of active disease, co-localising with activated ED1 positive macrophages infiltrating the retina. The aim of this study was to detect iNOS mRNA and investigate the expression of inflammatory cytokines, known to be important in the induction of iNOS *in vitro*, in diseased eyes throughout progression of EAU.

Method: EAU was induced in 10 week old male Lewis rats. Eyes were enucleated at days 9, 12, 14 and 18 post immunisation. Total RNA was isolated from each of the whole eyes using the RNAzol extraction kit and RT/PCR was subsequently used to detect iNOS, IL-1β, TNF-α, IFN-γ, IL-6 and GM-CSF gene expression.

Results: iNOS, IL-1β, TNF-α, IL-6 and GM-CSF were expressed throughout the course of the experiment. IFN-γ was not detected at days 9 or 18 but was strongly expressed at day 12 and to a lesser degree at day 14.

Conclusions: Expression of IFN-γ is upregulated during acute disease which coincides with enhanced production of iNOS by infiltrating macrophages. Therefore indicating the importance of IFN-γ, itself or in conjunction with other cytokines, in the regulation of iNOS expression not only *in vitro* but also *in vivo*.

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ROLE OF IMMUNOREGULATORY CYTOKINES TGF-β, IL-4, IL-10, IN THE IN VITRO PRODUCTION OF TNF AND NO BY RETINAL RESIDENT CELLS

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Purpose : We have previously shown that cultured retinal Müller glia (RMG) and retinal pigmented epithelium (RPE) isolated from Lewis rat and stimulated by lipopolysaccharide (LPS) and interferon (IFN)-γ release large amounts of tumor necrosis factor (TNF) and nitric oxide (NO). Ocular models of inflammation (S-antigen-induced experimental autoimmune uveoretinitis, EAU and endotoxin-induced uveitis, EIU, strongly implicate the involvement of TNF and NO in their pathogenesis. These multifunctional molecules have a key role in initiating and perpetuating local inflammatory response. The aim of this study was to determine the effect of the immunoregulatory cytokines interleukin (IL)-4, IL-10 and transforming growth factor (TGF)-β on the production of TNF and NO by RMG and RPE cells.
Methods : RMG and RPE cells cultured from retinas of young C3H/HeN mice, a strain susceptible to develop EIU. These two types of cells were treated *in vitro* with recombinant mouse IFN-γ, LPS from *Salmonella typhimurium*, recombinant mouse IL-4, IL-10 and human TGF-β1. Production of TNF (measured by L-929 bioassay) and NO (determined by nitrite release with Griess reaction) was examined in supernatants from RMG and RPE, 72 hrs after stimulation.
Results : We show that RMG cells failed to produce TNF when stimulated *in vitro* with LPS+IFN-γ while large amounts of TNF were detected in supernatants from stimulated RPE cells. Both cell types produced nitrite under LPS+ IFN-γ stimulation. Addition of TNF to LPS+IFN-γ did not further increase nitrite biosynthesis. Coincubating cells with TGF-β or L-10 with LPS+IFN-γ markedly reduced TNF and nitrite production. IL-4 was much less inhibitory.
Conclusion : Local synthesis of TGF-β and IL-10 may be important in regulating cytokine cascades and NO production. Administration of these regulatory cytokines could be effective therapies of ocular inflammation, preventing intra-retinal immune responses and photoreceptor necrosis.

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EFFECTS OF ALLOPURINOL AND STEROIDS ON OXIDATIVE TISSUE DAMAGE AND INFLAMMATORY PARAMETERS IN LENS-INDUCED UVEITIS

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Purpose: To evaluate the effects of allopurinol (Allo) on histological and biochemical changes in lens-induced uveitis (LIU) and to compare these effects with those of steroids (methylprednisolone=Pred) and a combination of both drugs (Allo/Pred).
Methods: Experiments were performed using 34 male Wistar rats. The animals were sensitized for eight weeks; thereafter, the anterior lens capsule was disrupted and therapy was started (Allo: 50 mg/kg bw; Pred: 7.5 mg/kg bw). Lipid peroxides (LPO) of the retinal tissue served as a parameter of oxidative tissue damage and were determined by two different methods (thiobarbituric acid assay and determination of malondialdehyde-like substances by HPLC). The myeloperoxidase (MPO) activity in the iris/ciliary body complex was analyzed spectrophotometrically for quantification of inflammation. Histological changes, e.g., accumulation of inflammatory cells in three morphological sections of iris and ciliary body of LIU eyes, were evaluated quantitatively.
Results: Allo led to a significant ($p<0.05$) reduction in LPO, MPO and the number of inflammatory cells evaluated histologically. The steroid showed only slight effects on LPO, whereas MPO and inflammatory cells were significantly ($p<0.05$) reduced. A combination of the two drugs showed a further (not significant) improvement over Allo alone.
Conclusions: The effects of allopurinol can be explained by its free radical scavenging activity and by the fact that allopurinol scavenges hypochlorous acid, which is produced via MPO catalyzation. The slight effect of Pred on LPO was not significant. Obviously, steroids predominantly suppress inflammation by influencing both the arachidonic acid pathway and the migration of macrophages and neutrophilic granulocytes to the inflamed tissue. The LPO results demonstrate that the free-radical scavenging activities of Pred play a minor role in this experimental model of uveitis.