

# **NF-*kappa* B**

## **5-1. Nuclear Factor-kappa B**

NF-kappa B has a gene network of at least 144 genes. These produce proteins relating to growth, oxidative stress, cyclo-oxygenase-2, chemokines, matrix metalloproteinases, VEGF and others:

NF-kappa B is activated in a number of ways, including free radical-induced oxidative stress, losses of glutathione, and changes in redox factors.

When NF-kappa B is activated within the RPE and other retinal cells, the adverse effects of growth factors, additional free radicals from NADPH-oxidase and from cyclo-oxygenase-2, the complex factors brought in by leucocytes responding to chemokines, the fragmentation of Bruch's membrane by matrix metalloproteinases, and finally the development of choroidal neovascularization (CNV) in response to VEGF (and a loss of PEDF when RPE die) begin to fill in the complex aspects of AMD as it develops over the years.

**Induction of angiogenic cytokine expression in cultured RPE by ingestion of oxidized photoreceptor outer segments.**

**Higgins GT, Wang JH, Dockery P, Cleary PE, Redmond HP.**

Department of Academic Surgery, University College, Cork, Ireland. garet@indigo.ie

**PURPOSE:** Normal aging is associated with accumulation of lipofuscin pigment in the retinal pigment epithelium (RPE). This may occur as a result of phagocytosis and incomplete degradation of oxidized photoreceptor outer segments (POS). This study was undertaken to determine whether phagocytosis of UV-irradiated POS (artificial lipofuscin) would increase expression in the RPE of various chemotactic and angiogenic cytokines. **METHODS:** ARPE-19 cells were exposed to latex beads (0.76 micro m), naive bovine POS, and UV-irradiated POS (Ox-POS;  $2 \times 10^7$ /mL), and supernatants were collected at 18 and 36 hours. The supernatants were assayed for IL-8, monocyte chemotactic protein-(MCP)-1, and TNF-alpha by ELISA. Protein synthesis and NFkappaB activity were inhibited by actinomycin D and SN50, respectively. Phagocytosis and generation of intracellular reactive oxygen species were assessed by flow cytometry. Confocal and electron microscopy studies were also performed to verify phagocytosis and cellular integrity. **RESULTS:** IL-8 and MCP-1 levels were decreased in the naive POS group (IL-8:  $473.76 \pm 66.9$  pg/mL,  $P = 0.0005$ ; MCP-1:  $550.1 \pm 21.8$  pg/mL,  $P = 0.0001$ ), but were increased in the Ox-POS group (IL-8:  $1348.8 \pm 164.9$  pg/mL; MCP-1:  $1772.28 \pm 65.19$  pg/mL) compared with the control (IL-8:  $741.09 \pm 39.8$  pg/mL; MCP-1:  $1413.47 \pm 38.4$  pg/mL) and latex bead groups (data not shown). TNF-alpha levels were not affected. At 12 hours (but not at 6 hours), ROS were increased in the Ox-POS group. The cytokine increases observed were dependent on de novo protein synthesis and were NF-kappaB dependent. **CONCLUSIONS:** Ingestion by RPE of oxidized bovine POS stimulates expression of the chemotactic and angiogenic factors IL-8 and MCP-1 that have the capability to promote angiogenesis directly, or indirectly through the accumulation of immune cells such as macrophages, which themselves may release angiogenic promoters and degrade Bruch's membrane. This may be of significance in the development of exudative AMD.

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## **Cyclooxygenase-2 gene expression and regulation in human retinal pigment epithelial cells.**

**Chin MS, Nagineni CN, Hooper LC, Detrick B, Hooks JJ.**

Virology and Immunology Section, Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892, USA.

**PURPOSE:** Cyclooxygenases (COX) orchestrate a variety of homeostatic processes and participate in various pathophysiological conditions. The retinal pigment epithelium (RPE) cell performs a variety of regulatory functions within the retina. The conditions under which COX-1 and COX-2 are expressed and upregulated in human RPE (HRPE) cells were determined. **METHODS:** COX gene expression was examined using RT-PCR analysis of untreated HRPE cultures or cultures exposed to bacterial lipopolysaccharide or various cytokines. COX proteins were detected by immunohistochemistry and Western blot analysis. Prostaglandin (PG) production was analyzed by EIA. **RESULTS:** Examination of untreated RPE cells revealed the presence of COX-2 mRNA and the absence of COX-1 mRNA. Moreover, cytokine stimulation more readily enhanced COX-2 gene expression than COX-1 gene expression. IL-1 beta, the most potent inducer of COX-2, also resulted in detection of COX-2 protein by immunocytochemical staining and Western blot analysis. There was a direct relationship between both the appearance and amount of COX-2 mRNA and protein synthesis and the degree of PG synthesis by RPE cells. Furthermore, COX inhibitors significantly decreased PG production. Pretreatment of RPE cells with a NF-kappa B inhibitor, PDTC, resulted in dose-dependent decrease in IL-1 beta-induced COX-2 gene expression and PG production. **CONCLUSIONS:** COX-2 was the predominant isoform of cyclooxygenase in untreated HRPE cells. When HRPE cells were treated with proinflammatory cytokines, COX-2 gene expression and synthesis of PGs were enhanced. NF-kappa B mediated the induction of COX-2 gene expression in HRPE cells. These studies indicate that RPE cells may participate in normal and pathologic retinal conditions through the induction of COX-2.

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**Effect of mutant IkappaB on cytokine-induced activation of NF-kappaB in cultured human RPE cells.**

**Yang P, McKay BS, Allen JB, Roberts WL, Jaffe GJ.**

Department of Ophthalmology, Duke University Medical Center, Durham, North Carolina 27710, USA.

**PURPOSE:** The nuclear transcription factor (NF)-kappaB is a central regulator of multiple inflammatory cytokines. The current study was conducted to determine whether infection of human retinal pigment epithelial (RPE) cells by adenovirus carrying a mutant inhibitory (I)-kappaB (IkappaB) transgene inhibits cytokine-induced activity of NF-kappaB and expression of NF-kappaB-dependent cytokines by preventing degradation of IkappaB. The persistence of recombinant protein expression and function after the viral infection was also examined. **METHODS:** Cultured human RPE cells were infected with adenovirus encoding either beta-galactosidase (LacZ) or mutant IkappaB and were treated with interleukin (IL)-1beta or tumor necrosis factor (TNF)-alpha. IkappaB protein expression was determined by Western blot. NF-kappaB nuclear translocation was evaluated by immunofluorescence, and functional NF-kappaB activation was determined by luciferase reporter assay. NF-kappaB-dependent cytokine gene expression was determined by reverse transcription-polymerase chain reaction. IL-1beta-induced monocyte chemoattractant protein (MCP)-1 protein secretion was measured by enzyme-linked immunosorbent assay. **RESULTS:** Stimulation of RPE cells with IL-1beta or TNF-alpha caused rapid degradation of the endogenous, but not mutant, IkappaB protein. Expression of the mutant IkappaB isoform inhibited cytokine-stimulated NF-kappaB nuclear translocation, NF-kappaB transcriptional activity, NF-kappaB-dependent gene expression, and secretion of MCP-1. Significant levels of mutant IkappaB protein were expressed for at least 7 weeks after infection. **CONCLUSIONS:** Infection of human RPE by an adenoviral vector carrying a mutant IkappaB transgene blocks NF-kappaB activation and expression of multiple NF-kappaB-dependent cytokine genes over an extended period. This technique will be useful to determine the role of NF-kappaB in experimental proliferative vitreoretinopathy (PVR), and may offer a novel approach to treatment of PVR with a gene therapy approach.

PMID: 12601067 [PubMed - indexed for MEDLINE]

**Modulation of matrix metalloproteinase and TIMP-1 expression by cytokines in human RPE cells.**

**Eichler W, Friedrichs U, Thies A, Tratz C, Wiedemann P.**

University of Leipzig, Eye Hospital, Leipzig, Germany. eichwolf@rz.uni-leipzig.de

**PURPOSE:** The balance between matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) is crucial for homeostasis of ocular extracellular matrices. To assess altered MMP activity as a determinant in the migration of human retinal pigment epithelial (RPE) cells, expression characteristics of several MMPs and TIMP-1 in RPE cell cultures were investigated. **METHODS:** Expression studies were performed with RT-PCR, ELISA, and immunofluorescence analysis. Secretion of MMP-2 was demonstrated by zymography. Migration of cytokine-stimulated RPE cells was evaluated with microporous membranes of permeable chambers. **RESULTS:** MMP-1, -2, -3, and -9; MT2-MMP; and TIMP-1 were expressed in cultured RPE cells. MMP-2 was detected on the cell surface and in secreted inactive and active forms. TGF-beta(2), IL-1beta, and TNF-alpha enhanced secretion of MMP-1, -2, and -3. TGF-beta(2) also stimulated MT2-MMP cell surface expression and release of TIMP-1. The mRNA levels of MMP-1, -2, and -3 and TIMP-1 were markedly increased by TNF-alpha and TGF-beta(2). MMP-2 mRNA levels were also upregulated by PDGF-BB. Migration of RPE cells stimulated by TGF-beta(2) or PDGF-BB was inhibited in presence of a synthetic MMP inhibitor. **CONCLUSIONS:** Proinflammatory cytokines and TGF-beta(2) play an important role in the upregulation of expression of MMP-1, -2, and -3 in RPE cells and account for a directional shift in the balance between MMPs and TIMPs. Facilitation of RPE cell migration stimulated by cytokines (i.e., TGF-beta(2) or PDGF-BB) in ocular diseases may be due to increased release of MMPs, in the presence of comparatively lower levels of their inhibitors.

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**Expression of LRP1 in retinal pigment epithelial cells and its regulation by growth factors.**

**Hollborn M, Birkenmeier G, Saalbach A, Iandiev I, Reichenbach A, Wiedemann P, Kohen L.**

Department of Ophthalmology, Paul Flechsig Institute for Brain Research, University of Leipzig, Leipzig, Germany. hollbm@medizin.uni-leipzig.de

**PURPOSE:** The retinal pigment epithelial (RPE) cells are mitotically inactive under normal conditions, but play a pivotal role in the pathogenesis of proliferative vitreoretinopathy (PVR). Triggered by changes in the concentrations of growth factors, RPE cells reenter the cell cycle, proliferate, and migrate onto the retinal surface, into the subretinal space, and into the vitreous. The receptor for alpha(2)-macroglobulin (low-density lipoprotein receptor-related protein [LRP1], or CD91) is known to be involved in the processes of cell migration and invasion, as well as in the regulation of growth factor homeostasis. The purpose of this study was to investigate the expression of this receptor and its regulation, at the protein and mRNA levels, in human (h)RPE cells. **METHODS:** The cell surface expression of the receptor was studied by immunocytochemistry and flow cytometry. The endocytosis-related activity of LRP1 in hRPE cells was examined by assessing the uptake of FITC-labeled, methylamine (MA)-treated alpha(2)-M (alpha(2)-M-MA). LRP1 mRNA expression was analyzed by means of the RNase protection assay (RPA) after the hRPE cells were stimulated with the growth factors TGF-beta1, TGF-beta2, PDGF, VEGF (each 10 ng/mL), or bFGF (5 ng/mL). **RESULTS:** hRPE cells expressed LRP1 on their cell surface. The receptor mediated rapid binding and endocytosis of FITC-labeled alpha(2)-M-MA. The expression of LRP1 mRNA strongly increased on stimulation of the cells with TGF-beta1, TGF-beta2, or VEGF, whereas PDGF or bFGF elicited only minor effects. **CONCLUSIONS:** The expression of functionally active LRP1 in hRPE cells suggests that the receptor may be involved in cell migration and invasion, as reported for other LRP1-expressing cells. Thus, certain growth factors may control RPE cell migration and invasion in vivo through a regulation of LRP1 expression. As LRP1 mediates the clearance of alpha(2)-M, known to regulate the homeostasis of many cytokines and growth factors, this receptor may be a promising target for therapeutic intervention in PVR.

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**N(epsilon)(carboxymethyl)lysine and the AGE receptor RAGE colocalize in age-related macular degeneration.**

**Hammes HP, Hoerauf H, Alt A, Schleicher E, Clausen JT, Bretzel RG, Laqua H.**

Third Medical Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany.

**PURPOSE:** To investigate whether glycoxidation products and the receptor for advanced glycation end products (RAGE) are present and colocalize in subfoveal membranes of patients with age-related macular degeneration (ARMD). **METHODS:** Surgically removed subfoveal fibrovascular membranes from 12 patients, 11 related to ARMD and 1 to an idiopathic membrane, were analyzed for the presence of the glycoxidation product N(epsilon)-(carboxymethyl)lysine (CML), one of the receptors for advanced glycation end products, RAGE, and the activation of NFkappaB, using immunohistochemistry. **RESULTS:** CML-like immunoreactivity was found in all ARMD specimens examined adjacent or colocalized with RAGE, but not in the idiopathic membrane. RAGE immunoreactive material was found in CD68-positive cells and in the fibrous matrix. CD68-positive cells and surrounding areas stained for p50, the activated form of NFkappaB. **CONCLUSIONS:** These results indicate that glycoxidation products are present in subretinal membranes of patients with ARMD. The concomitant expression of RAGE in these membranes and the finding of activated NFkappaB is suggestive of an implication of glycoxidation product formation in the pathogenesis of the disease.

PMID: 10393061 [PubMed - indexed for MEDLINE]

**The receptor for advanced glycation end products is induced by the glycation products themselves and tumor necrosis factor-alpha through nuclear factor-kappa B, and by 17beta-estradiol through Sp-1 in human vascular endothelial cells.**

**Tanaka N, Yonekura H, Yamagishi S, Fujimori H, Yamamoto Y, Yamamoto H.**

Department of Biochemistry and the Department of Ophthalmology, Kanazawa University School of Medicine, Japan.

The binding of advanced glycation end products (AGE) to the receptor for AGE (RAGE) is known to deteriorate various cell functions and is implicated in the pathogenesis of diabetic vascular complications. Here we show that AGE, tumor necrosis factor-alpha (TNF-alpha), and 17beta-estradiol (E(2)) up-regulated RAGE mRNA and protein levels in human microvascular endothelial cells and ECV304 cells, with the mRNA stability being essentially invariant. Transient transfection experiments with human RAGE promoter-luciferase chimeras revealed that the region from nucleotide number -751 to -629 and the region from -239 to -89 in the RAGE 5'-flanking sequence exhibited the AGE/TNF-alpha and E(2) responsiveness, respectively. Site-directed mutation of an nuclear factor-kappaB (NF-kappaB) site at -671 or of Sp-1 sites at -189 and -172 residing in those regions resulted in an abrogation of the AGE/TNF-alpha- or E(2)-mediated transcriptional activation. Electrophoretic mobility shift assays revealed that ECV304 cell nuclear extracts contained factors which retarded the NF-kappaB and Sp-1 elements, and that the DNA-protein complexes were supershifted by anti-p65/p50 NF-kappaB and anti-Sp-1/estrogen receptor alpha antibodies, respectively. These results suggest that AGE, TNF-alpha, and E(2) can activate the RAGE gene through NF-kappaB and Sp-1, causing enhanced AGE-RAGE interactions, which would lead to an exacerbation of diabetic microvasculopathy.

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**Role of NF-kappaB-mediated interleukin-8 expression in intraocular neovascularization.**

**Yoshida A, Yoshida S, Khalil AK, Ishibashi T, Inomata H.**

Department of Ophthalmology, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

**PURPOSE:** To investigate the role of interleukin (IL)-8 in intraocular neovascularization and the mechanism of its production. **METHODS:** Interleukin-8 was measured with enzyme-linked immunosorbent assays in vitreous and aqueous fluid obtained from patients with neovascular diseases. Localization of IL-8 was examined by immunohistochemistry. An in vitro angiogenesis assay was performed on collagen gels, by using bovine aortic endothelial cells to determine the effect of the vitreous fluid. In bovine retinal glial cells under hypoxia, NF-kappaB activation was evaluated by immunoblot analysis and by electrophoretic mobility shift assay, and IL-8 and vascular endothelial growth factor (VEGF) mRNA expression was determined by semiquantitative reverse transcription-polymerase chain reaction. **RESULTS:** The concentration of IL-8 in vitreous fluid of patients with retinal neovascularization was significantly higher than that of patients without neovascular disease. Interleukin-8 immunostaining was detected in vascular endothelial cells and glial cells in the retinas with neovascularization. Vitreous fluid with high concentrations of IL-8 induced tubular morphogenesis in endothelial cells, and this effect was inhibited to a similar extent by neutralizing antibodies to IL-8 or to VEGF. In glial cells, in vitro, hypoxia induced NF-kappaB activation and increased IL-8 and VEGF mRNA. Furthermore, pyrrolidine dithiocarbamate, a specific inhibitor of NF-kappaB activation, prevented the induction of the IL-8 gene, but not that of the VEGF gene. **CONCLUSIONS:** These results suggest that IL-8 induced by hypoxia and mediated by NF-kappaB may contribute to the pathogenesis of intraocular neovascularization.

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## **Coordinate activation of HIF-1 and NF-kappaB DNA binding and COX-2 and VEGF expression in retinal cells by hypoxia.**

**Lukiw WJ, Ottlecz A, Lambrou G, Grueninger M, Finley J, Thompson HW, Bazan NG.**

Neuroscience Center, Louisiana State University Health Sciences Center, New Orleans, Louisiana 70112, USA. wlukiw@lsuhsc.edu

**PURPOSE.** Proinflammatory signaling mechanisms are implicated in the induction of retinal neovascularization (NV) during ischemic retinopathies. This study examined transcription factor (TF) AP-1, HIF-1, and NF-kappaB DNA-binding in relation to cyclooxygenase (COX)-2 and VEGF RNA and protein levels in hypoxia-triggered monkey choroidal retinal (RF/6A) endothelial cells. Effects of the carboxamide CGP43182 were tested on COX-2 and VEGF activation and prostaglandin (PGE)(2) release. **METHODS.** RF/6A cells were subjected to hypoxia for 1 and 3 hours, at which times RNA and proteins were isolated. Potential AP-1, hypoxia-inducible factor (HIF)-1 and NF-kappaB DNA-binding sites were identified using DNA sequence search algorithms and were analyzed using gel-shift assay. COX-2 and VEGF RNA, protein, and PGE(2) levels were quantified by RT-PCR, Western analysis, and enzyme immunoassay, respectively. Tubular morphogenesis was analyzed with phase-contrast imaging microscopy. **RESULTS.** Nuclear AP-1, HIF-1 and NF-kappaB promoter DNA binding increased 1.5-, 4-, and 3-fold, respectively, after 1 hour of hypoxia. COX-2 RNA was elevated five- and fourfold after 1 and 3 hours of hypoxia, respectively. VEGF RNA and protein abundance lagged behind COX-2 induction but were each increased two- to threefold 3 hours after hypoxia. CGP43182 was found to inhibit NF-kappaB DNA binding, COX-2 and VEGF gene expression, PGE(2) release, and hypoxia-induced tubular morphogenesis. **CONCLUSIONS.** Maximum HIF-1 and NF-kappaB DNA binding immediately before COX-2 expression suggests that these TFs are important regulators of COX-2 induction in hypoxic RF/6A cells. IL-1beta emulated AP-1, HIF-1, and NF-kappaB DNA binding during hypoxia and may be a novel cytokine trigger for NV. CGP43182 appears to be an effective inhibitor of NV. VEGF expression appears to be regulated through dual interdependent mechanisms involving HIF-1 directly and indirectly through NF-kappaB-mediated COX-2 expression and PGE(2) production.

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**Hypoxia activates matrix metalloproteinase expression and the VEGF system in monkey choroid-retinal endothelial cells: Involvement of cytosolic phospholipase A2 activity.**

**Ottino P, Finley J, Rojo E, Ottlecz A, Lambrou GN, Bazan HE, Bazan NG.**

Neuroscience Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA 70112, USA.

**PURPOSE:** To determine whether the gene expression of matrix metalloproteinases (MMPs) as well as that of the pro-angiogenic cytokine vascular endothelial growth factor (VEGF) and its receptors change in response to hypoxic exposure in a primate choroid-retinal endothelial cell line, and furthermore, whether cytosolic phospholipase A2 (cPLA2) plays a role in this process. **METHODS:** Rhesus macaque choroid-retinal endothelial (RF/6A) cells were incubated under hypoxic conditions for 1, 2, 4, or 8 h prior to RNA extraction. In some experiments cells were pretreated with the cPLA2 inhibitor AACOCF3 (10 microM) for 30 min prior to hypoxia. Changes in gene expression were determined by RT-PCR and quantified by real-time PCR for urokinase plasminogen activator (uPA), collagenase-1 (MMP-1), membrane type-1 metalloproteinase (MT1-MMP), gelatinases A and B (MMP-2, MMP-9), tissue inhibitor-2 (TIMP-2), VEGF and its receptors, Flt-1 (VEGFR-1), KDR (VEGFR-2), and neuropilin-1 (NP-1). MMP-2 secreted by the cells was evaluated by zymography. VEGF release was measured by ELISA. In tube-formation studies, endothelial cells (EC) were seeded into collagen gel, exposed to hypoxia for 4 h, then incubated under normoxic conditions for 72 h. **RESULTS:** Hypoxia triggered a three fold increase in the gene expression of MT1-MMP, MMP-2, and TIMP-2, and a ten fold increase in MMP-2 levels. Moreover it also induced tube formation in EC. Expression of uPA, MMP-1, and MMP-9 mRNA was not detected. Pretreatment with AACOCF3 abolished hypoxia-induced tube formation and MT1-MMP, MMP-2, and TIMP-2 transcription. Furthermore, hypoxia produced a significant, sustained increase in the gene expression and release of VEGF-165, the only VEGF-A isoform detected in these cells. AACOCF3 reduced the hypoxia-induced VEGF release at 8 h of hypoxia. VEGF receptors KDR and NP-1 were constitutively expressed in EC and up-regulated under hypoxic conditions. **CONCLUSIONS:** In monkey choroid-retinal EC, hypoxia selectively induces MMP-2 activity. This induction is preceded by MT1-MMP, MMP-2, and TIMP-2 mRNA expression, as well as that of the VEGF-165 isoform and its receptors KDR and NP1. These increases possibly result from hypoxia-induced activation of cPLA2 and subsequent release of arachidonic acid and its conversion to prostaglandins. These molecular changes in EC could, in part, contribute to the angiogenic response that occurs in the development of ischemic retinopathies and choroidal neovascularization.

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**Pigment epithelium-derived factor suppresses ischemia-induced retinal neovascularization and VEGF-induced migration and growth.**

**Duh EJ, Yang HS, Suzuma I, Miyagi M, Youngman E, Mori K, Katai M, Yan L, Suzuma K, West K, Davarya S, Tong P, Gehlbach P, Pearlman J, Crabb JW, Aiello LP, Campochiaro PA, Zack DJ.**

Department of Ophthalmology, Johns Hopkins University School of Medicine, Maumenee 809, 600 N. Wolfe Street, Baltimore, MD 21287, USA.

**PURPOSE:** To determine the effect of pigment epithelium-derived factor (PEDF) in a mouse model of ischemia-induced retinal neovascularization and on vascular endothelial growth factor (VEGF)--induced migration and growth of cultured microvascular endothelial cells. **METHODS:** Human recombinant PEDF was expressed in the human embryonic kidney 293 cell line and purified by ammonium sulfate precipitation and cation exchange chromatography. C57BL/6 mice were exposed to 75% oxygen from postnatal day (P)7 to P12 and then returned to room air. Mice received intravitreal injections of 2 microg PEDF in one eye and vehicle in the contralateral eye on P12 and P14. At P17, mice were killed and eyes enucleated for quantitation of retinal neovascularization. The mitogenic and motogenic effects of VEGF on cultured bovine retinal and adrenal capillary endothelial cells were examined in the presence or absence of PEDF, using cell counts and migration assays. **RESULTS:** Two species of human recombinant PEDF, denoted A and B, were purified to apparent homogeneity. PEDF B appeared to comigrate on SDS-PAGE with PEDF from human vitreous samples. Changes in electrophoretic mobility after peptide-N-glycosidase F (PNGase F) digestion suggest that both PEDF forms contain N-linked carbohydrate. Analyses of the intact proteins by liquid chromatography-electrospray mass spectrometry (LC-ESMS) revealed the major molecular weight species for PEDF A (47,705 +/- 4) and B (46,757 +/- 5). LC-ESMS analysis of tryptic peptides indicated that PEDF A and B exhibit differences in glycopeptides containing N-acetylneuraminic acid (NeuAc) and N-acetylhexosamine (HexNAc). Intravitreal administration of either species of PEDF significantly inhibited retinal neovascularization (83% for PEDF A and 55% for PEDF B; P = 0.024 and 0.0026, respectively). PEDF A and B (20 nM) suppressed VEGF-induced retinal microvascular endothelial cell proliferation by 48.8% and 41.4%, respectively, after 5 days (P < 0.001) and VEGF-induced migration by 86.5% +/- 16.7% and 78.1% +/- 22.3%, respectively, after 4 hours (P = 0.004 and P = 0.008, respectively). **CONCLUSIONS:** These data indicate that elevated concentrations of PEDF inhibit VEGF-induced retinal endothelial cell growth and migration and retinal neovascularization. These findings suggest that localized administration of PEDF may be an effective approach for the treatment of ischemia-induced retinal neovascular disorders.

PMID: 11867604 [PubMed - indexed for MEDLINE]

**Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress.**

**Shono T, Ono M, Izumi H, Jimi SI, Matsushima K, Okamoto T, Kohno K, Kuwano M.**

Department of Biochemistry, Kyushu University School of Medicine, Fukuoka, Japan.

Oxygen radicals are induced under various pathologic conditions associated with neovascularization. Oxygen radicals modulate angiogenesis in cultured human microvascular endothelial cells by an unknown mechanism. Treatment of human microvascular endothelial cells for 15 min with 0.1 to 0.5 mM hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or 100 U of tumor necrosis factor alpha per ml induced tubular morphogenesis in type I collagen gels. Gel shift assays with nuclear extracts demonstrated that H<sub>2</sub>O<sub>2</sub> increases the binding activities of two transcription factors, NF-kappaB and AP-1, but not of Sp1. Tumor necrosis factor alpha increased the binding activities of all three factors. A supershift assay with specific antibodies against JunB, JunD, and c-Jun (Jun family) showed that the antibody against c-Jun supershifted the AP-1 complex after H<sub>2</sub>O<sub>2</sub> treatment. Coadministration of the antisense sequence of NF-kappaB inhibited H<sub>2</sub>O<sub>2</sub>-dependent tubular morphogenesis, and the antisense c-Jun oligonucleotide caused partial inhibition. The angiogenic factor responsible for H<sub>2</sub>O<sub>2</sub>-induced tubular morphogenesis was examined. Cellular mRNA levels of vascular endothelial growth factor and interleukin-8 (IL-8), but not those of transforming growth factor alpha, were increased after treatment with 0.5 mM H<sub>2</sub>O<sub>2</sub>. Coadministration of anti-IL-8 antibody inhibited tubular morphogenesis enhanced by H<sub>2</sub>O<sub>2</sub>, and IL-8 itself also enhanced the formation of tube-like structures. Treatment with antisense NF-kappaB oligonucleotide completely blocked H<sub>2</sub>O<sub>2</sub>-dependent IL-8 production by endothelial cells. The tubular morphogenesis of vascular endothelial cells after treatment with oxidative stimuli and its possible association with NF-kappaB and IL-8, is examined.

PMID: 8754823 [PubMed - indexed for MEDLINE]

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## **NF-kappaB activation in light-induced retinal degeneration in a mouse model.**

**Wu T, Chen Y, Chiang SK, Tso MO.**

Wilmer Eye Institute, Johns Hopkins School of Medicine, Baltimore, Maryland 21287, USA.

**PURPOSE:** To investigate the modulation of nuclear factor (NF)-kappaB in light-induced photoreceptor degeneration in a mouse model. **METHODS:** Mice were exposed to intense green light. Light-induced activation of NF-kappaB and its nuclear localization were studied by immunohistochemistry. The NF-kappaB DNA-binding activity in the retinas after exposure to light was measured by electrophoretic mobility shift assay (EMSA). Nuclear transactivation of NF-kappaB in the photoreceptor cells was determined by quantitative real-time (qRT)-PCR. The amount of NF-kappaB p65 in the photoreceptor cells after exposure to light was assessed by Western blot analysis. To obtain more photoreceptor-specific information, microdissected photoreceptor cells were used in some studies. **RESULTS:** By an immunohistochemical method, the perinuclear region of the photoreceptor cells was heavily labeled with an antibody to activated NF-kappaB after a 1-hour exposure to light. Nuclear localization of NF-kappaB in the photoreceptor nucleus was seen at 12 hours. In the experiments involving 3 hours of exposure to light followed by recovery in the dark, nuclear localization of NF-kappaB was also noted after 12 hours' recovery in the dark. During continuous exposure to light, the NF-kappaB DNA-binding activity gradually increased and reached its maximum at 12 hours. There was an increase of NF-kappaB p65 protein at 3 hours. The mRNA levels of IkappaBalpha were upregulated after 6 hours' exposure to light. **CONCLUSIONS:** Intense light activated NF-kappaB in the photoreceptor cells in vivo, increased the NF-kappaB DNA-binding activity, and increased the expression of mRNA of IkappaBalpha, a target gene of NF-kappaB.

PMID: 12202499 [PubMed - indexed for MEDLINE]

Diabetes. 2002 Jul;51(7):2241-8.

**Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes.**

**Romeo G, Liu WH, Asnaghi V, Kern TS, Lorenzi M.**

Schepens Eye Research Institute, Harvard Medical School, 20 Staniford Street, Boston, MA 02114, USA.

To reconstruct the events that may contribute to the accelerated death of retinal vascular cells in diabetes, we investigated in situ and in vitro the activation of nuclear factor-kappaB (NF-kappaB), which is triggered by cellular stress and controls several programs of gene expression. The retinal capillaries of diabetic eye donors showed an increased number of pericyte nuclei positive for NF-kappaB, when compared with nondiabetic donors, whereas endothelial cells were negative. Microvascular cell apoptosis and acellular capillaries were increased only in the diabetic donors with numerous NF-kappaB-positive pericytes. Likewise, high glucose in vitro activated NF-kappaB in retinal pericytes but not in endothelial cells, and increased apoptosis only in pericytes. Studies with NF-kappaB inhibitors suggested that in pericytes, basal NF-kappaB has prosurvival functions, whereas NF-kappaB activation induced by high glucose is proapoptotic. Pericytes exposed to high glucose showed increased expression of Bax and of tumor necrosis factor-alpha, which were prevented by the NF-kappaB inhibitors and mimicked by transfection with the p65 subunit of NF-kappaB, and failed to increase the levels of the NF-kappaB-dependent inhibitors of apoptosis. Colocalization of activated NF-kappaB and Bax overexpression was observed in the retinal pericytes of diabetic donors. A proapoptotic program triggered by NF-kappaB selectively in retinal pericytes in response to hyperglycemia is a possible mechanism for the early demise of pericytes in diabetic retinopathy.

PMID: 12086956 [PubMed - indexed for MEDLINE]

**Suppression of NF-kappaB-dependent proinflammatory gene expression in human RPE cells by a proteasome inhibitor.**

**Wang XC, Jobin C, Allen JB, Roberts WL, Jaffe GJ.**

Department of Ophthalmology, Duke University Medical Center, Durham, North Carolina, USA.

**PURPOSE:** To determine whether nuclear transcription factor-kappaB (NF-kappaB) is activated in human retinal pigment epithelial (hRPE) cells in response to interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNF-alpha), or interferon-gamma (IFN-gamma) alone or in combination and if so, whether expression of proinflammatory genes induced by these agents can be blocked by a proteasome inhibitor, MG-132, which inhibits the degradation of I kappaB, an NF-kappaB inhibitor, thereby preventing nuclear translocation of NF-kappaB. **METHODS:** Cultured hRPE were pretreated for 60 minutes with medium alone or medium containing the proteasome inhibitor MG-132 (20 microM) and then exposed to TNF-alpha (1.1 x 10<sup>3</sup> U/ml), IL-1beta (5 U/ml), or IFN-gamma (7.5 x 10<sup>3</sup> U/ml) alone or in combination (TII). Nuclear translocation of NF-kappaB was determined by fluorescence staining of the NF-kappaB Rel A (p65) subunit. Cytoplasmic I kappaB protein was measured by western blot analysis. Nuclear extract binding to kappaB DNA motifs was measured by electrophoretic mobility shift assay and antibody supershift assay. Steady state mRNA expression of the chemokines melanoma growth stimulating activity (MGSA)/gro-alpha, regulated on activation normal T-cell expression and secreted (RANTES), and monocyte chemoattractant protein (MCP-1), the cytokines IL-1beta and macrophage colony stimulating factor (M-CSF) and intercellular adhesion molecule-1 (ICAM-1) was evaluated by semiquantitative reverse transcription-polymerase chain reaction. Chemokine and cytokine protein secretion was measured by enzyme-linked immunosorbent assay. Cell-surface ICAM-1 expression was determined by flow cytometry. **RESULTS:** TNF-alpha, IL-1beta, and TII but not IFN-gamma alone caused degradation of I kappaB, Rel A nuclear translocation, and increased NF-kappaB DNA binding activity, effects that were blocked by pretreatment with MG-132. MG-132 suppressed MGSA/gro-alpha, RANTES, MCP-1, IL-1beta, M-CSF, and ICAM-1 mRNA expression and secreted RANTES, MCP-1, and M-CSF protein, and cell-surface ICAM-1 that were induced by IL-1beta, TNF-alpha, and TII. **CONCLUSIONS:** TNF-alpha, IL-1beta, and TII induce expression of proinflammatory cytokines and ICAM-1 in hRPE cells through an NF-kappaB-dependent signal transduction pathway. This effect is blocked by MG-132, a proteasome inhibitor that prevents I kappaB degradation. Inhibition of NF-kappaB may be a useful strategy to treat proliferative vitreoretinopathy and uveitis, ocular diseases initiated and perpetuated by cytokine activation.

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**Suppression of retinal neovascularization by the NF-kappaB inhibitor pyrrolidine dithiocarbamate in mice.**

**Yoshida A, Yoshida S, Ishibashi T, Kuwano M, Inomata H.**

Department of Ophthalmology, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

**PURPOSE:** To evaluate the effect of pyrrolidine dithiocarbamate (PDTC), an inhibitor of nuclear factor kappaB (NF-kappaB), on retinal neovascularization in a murine model of ischemic retinopathy. **METHODS:** One-week-old C57BL/6N mice were exposed to 75%+/-2% oxygen for 5 days and then were returned to room air to induce retinal neovascularization. After the return to room air, the left and right eyes were injected intravitreally with PDTC or a vehicle, respectively. Retinal neovascularization was examined by injecting fluorescein dextran and angiography after 5 days in room air and was quantitated histologically with a masked protocol. The effects of PDTC on NF-kappaB activation were evaluated by immunohistochemistry. To examine the toxicity of PDTC, the histologic change in the retina was examined by light and electron microscopy. **RESULTS:** Retinal neovascularization in the eye injected with PDTC by intravitreal methods was reduced in 100% of animals compared with that apparent in the vehicle-treated eye. The inhibitory effect was dose-dependent, with a maximal inhibition of 39% ( $P < 0.01$ ) at a dose of 1 nmole. The immunostaining intensity for NF-KB in the retina was reduced by PDTC injections. No side effects by PDTC in the retina were observed by light and electron microscopy. **CONCLUSIONS:** NF-kappaB activation appears to be required for retinal angiogenesis, given that the administration of PDTC suppressed retinal neovascularization. PDTC may prove beneficial in the treatment of ischemic neovascular diseases such as diabetic retinopathy and retinal vein occlusion.

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