CD40 Mediates Retinal Inflammation and Neurovascular Degeneration


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CD40 Mediates Retinal Inflammation and Neurovascular Degeneration


Retinopathies are major causes of visual impairment. We used a model of ischemic retinopathy to examine the role of CD40 in the pathogenesis of retinal injury. Retinal inflammation, loss of ganglion cells, and capillary degeneration were markedly attenuated in ischemic retinas of CD40−/− mice. Up-regulation of NOS2 and COX2 after retinal ischemia were blunted in CD40−/− mice. NOS2-COX-2 up-regulation in ischemic retinas from wild-type mice was at least in part explained by recruitment of NOS2+COX-2+ leukocytes. Up-regulation of KC/CXCL1 and ICAM-1 also required CD40. Retinal endothelial and Muller cells expressed CD40. Stimulation of these cells through CD40 caused ICAM-1 up-regulation and KC/CXCL1 production. Bone marrow transplant experiments revealed that leukocyte infiltration, ganglion cell loss, and up-regulation of proinflammatory molecules after retinal ischemia were dependent on CD40 expression in the retina and not peripheral blood leukocytes. These studies identified CD40 as a regulator of retinal inflammation and neurovascular degeneration. They support a model in which CD40 stimulation of endothelial and Muller cells triggers adhesion molecule up-regulation and chemokine production, promoting the recruitment of leukocytes that express NOS2/COX-2, molecules linked to neurovascular degeneration. The Journal of Immunology, 2008, 181: 8719–8726.

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2 Abbreviations used in this paper: NOS2, NO synthase 2; COX-2, cyclooxygenase-2; EGFP, enhanced green fluorescent protein; IOP, intraocular pressure; I/R, ischemia/reperfusion; KO, knockout; cMFI, corrected mean fluorescence intensity.

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CD40 promotes leukocyte infiltration, ganglion cell loss, and capillary degeneration after retinal I/R. One eye from each B6 and CD40−/− mouse was subjected to I/R. Nonischemic eyes were used as controls (Ctr). Eyes were obtained 2 days (A and B) and 8 days (C) after I/R. A, Leukocytes in the retina and vitreous were noted in ischemic eyes from B6 mice (arrowheads) but not from CD40−/− mice. A marked decrease in the number of ganglion cells is observed in ischemic eyes from B6 (arrow) but not from CD40−/− mice. Original magnification, ×400. B, The number of ganglion cells per 100 μm was significantly higher in ischemic CD40−/− than B6 mice. C, Fold increase in the numbers of acellular capillaries between ischemic (red) and nonischemic (white) retinas was determined as described in Materials and Methods (1, 27). Three to five mice per group.

Animals were euthanized 1, 2, or 8 days after I/R injury as indicated. Studies were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University School of Medicine.

Bone marrow transplantation

This was conducted as previously described (26). Briefly, bone marrow cells were obtained from the femur and tibia of B6 EGFP or CD40−/− mice. Recipient B6 and CD40−/− mice received 12 Gy of whole-body irradiation in two doses 3 h apart followed by i.v. administration of 4 × 106 bone marrow cells. Mice were used for experiments at 4 wk posttransplant. The success of the transplant was assessed at 4 wk by examining the percentage of peripheral blood leukocytes that express EGFP. Administration of EGFP B6 bone marrow cells to CD40−/− (knockout; KO) mice (B6→KO) and of CD40−/− bone marrow cells to EGFP B6 mice (KO→B6) resulted in >90% EGFP+ and >90% EGFP− leukocytes in peripheral blood respectively.

Histopathology and quantitation of degenerate (acellular) capillaries

Histological changes induced by I/R were assessed as described before (1). Formalin-fixed, paraffin-embedded sections were stained with periodic acid-Schiff and hematoxylin for light microscopy. The ganglion cell nuclei and leukocytes were counted under 400X. Retinal vasculatures were isolated as we previously described (27, 28). Briefly, freshly isolated eyes were fixed with 10% neutral buffered formalin for 1 day. Retinas were isolated, washed in water overnight, and then incubated with 3% crude trypsin (Difco; BD Biosciences) at 37°C for 1 h. Nonvascular cells were gently brushed away from the vasculature, and the isolated vasculature laid out on slides and used for acellular capillary examination. Sections were then stained with periodic acid-Schiff and hematoxylin. Acellular capillaries were identified as capillary-sized vessel tubes having no nuclei anywhere along their length and are reported per square millimeter of retinal area (1, 27).

Immunohistochemistry

Eyes were fixed overnight in 4% paraformaldehyde in phosphate buffer and embedded in paraffin. Sections were deparaffinized and subjected to Ag retrieval by heating in citrate buffer with a microwave for 15 min. Sections were permeabilized with 0.1% Triton X-100. After blocking for 1 h with PBS containing 5% normal goat serum, sections were incubated overnight at 4°C with anti-NOS2 or anti-COX-2 Ab (Millipore and Cayman Chemical Co., respectively) followed by incubation with secondary Ab conjugated to biotin (Jackson ImmunoResearch Laboratories) for 1 h at room temperature. Sections were resolved using a Vectastain ABC kit (Vector Laboratories) followed by diaminobenzidine substrate and counterstaining with hematoxylin. Specificity of staining was determined by incubating sections with control primary Ab plus secondary Ab or with secondary Ab alone.

Real-time quantitative RT-PCR

Total cellular RNA from retinas was isolated using the RNeasy kit (Qiagen). After treatment with DNase (Ambion), 0.5 μg of RNA was used to generate cDNA using oligo(dT)12-18 primers and Superscript III reverse transcriptase (Invitrogen Life Technologies) as we described (29). cDNA (2.5 μl) was used as template for RT-PCR using SYBR Green PCR Master Mix (Applied Biosystems) and 20 pM (each) primers in a final volume of 50 μl. Primer sequences for NOS2 (30), COX-2 (31), ICAM-1 (30), keratinocyte-derived chemokine (KC)/CXCL1 (32), MCP-1 (33), and 18S rRNA (29) were previously described. Expression of these genes was assessed using a 7300 Real Time PCR System (Applied Biosystems). Each cDNA sample was run in duplicate. Samples were normalized according to the content of 18S rRNA (29).

Isolation of primary retinal cells

Mice were anesthetized and eyes were enucleated. Retinas were isolated and digested in a solution containing papain, 15 IU/ml, and DNase, 15 μg/ml (Worthington Biochemicals) for 30 min at 37°C. Tissue was dissociated by gentle pipetting and passed through a 40-μm pore size cell strainer. Flow through was mixed with FBS and washed. Tissue trapped by the strainer was digested with collagenase type I (1 mg/ml; Worthington Biochemicals) for 30 min at 37°C to free endothelial cells. After dissociation, mixing with FBS and washing, cells obtained after papain-DNase and collagenase treatment were pooled and counted. Viability of the cells was consistently >90% as assessed by trypan blue exclusion.

Retinal cell lines and CD40 stimulation

A line of mouse retinal endothelial cells and a line of mouse retinal glial cells with Muller cell characteristics (34, 35) were gifts from Dr. N. Sheibani (University of Wisconsin, Madison, WI). Cells were maintained as described at 33°C in DMEM containing 20% FBS (HyClone), 2 mM L-glutamine, 2 mM sodium pyruvate, 20 mM HEPES, 1% nonessential amino acids, 100 μg/ml streptomycin, 100 U/ml penicillin, endothelial
growth supplement (100 μg/ml; Sigma-Aldrich), and IFN-γ (44 U/ml; PeproTech). Cells were treated with or without mouse CD154 (1/4 dilution of cell-free supernatants, a gift from Dr. R. Kornbluth, University of California San Diego; Ref. 36) or IL-1β (25 pg/ml; PeproTech) for 24 h at 37°C. Cells were used for flow cytometry, and cell supernatants were collected for ELISA.

**Flow cytometry**

Suspensions of primary retinal cells were incubated with Fc block (BD Biosciences) followed by staining with anti-CD40 PE (BD Biosciences) and biotinylated anti-CD105 mAb followed by streptavidin-allophycocyanin (eBioscience) or appropriate isotype control mAbs. For detection of intracellular markers, cells were then fixed and permeabilized by using IntraPrep permeabilization reagent (Beckman Coulter) following the manufacturer’s instructions (37). Thereafter, cells were stained with anti-vimentin-FITC (Santa Cruz Biotechnologies), anti-rhodopsin mAb (ID4, a gift from Dr. K. Palczeweski, Case Western Reserve University) followed by allophycocyanin-conjugated secondary Ab (eBioscience) or appropriate isotype control Abs. After fixation with 1% paraformaldehyde, cells were analyzed using a LSR II (BD Biosciences). Expression of CD40 (corrected mean fluorescence intensity; cMFI) was analyzed on gated CD105+ vimentin+ cells, or rhodopsin+ cells. Retinal cell lines were stained with anti-CD40 allophycocyanin, anti-ICAM-1 PE or isotype control mAbs (eBiosciences).

**ELISA**

 Supernatants from retinal cell lines cultured in 96-well plates were collected 24 h after addition of either mouse CD154 or IL-1β. Cell-free supernatants were used to measure concentrations of KC/CXCL1 (R&D Systems). The lower limit of detection of the ELISA was 2 pg/ml.

**Statistical analysis**

All results were expressed as the mean ± SEM. Data were analyzed by a two-tailed Student t test, ANOVA, and the nonparametric Kruskal-Wallis test followed by the Mann-Whitney U test. Differences were considered statistically significant at p < 0.05.

**Results**

**CD40 is an important mediator of inflammation and neurovascular degeneration after retinal I/R**

We used a well-established model of I/R-induced retinal injury (transient elevation of IOP) (1) to examine the in vivo relevance of CD40. The histological features of the retina from untreated B6 and CD40−/− mice were similar (Fig. 1A). Histological examination 2 days after I/R revealed the presence of leukocytes infiltrating in the inner layers of retinas and vitreous from B6 mice (Fig. 1A; see also Fig. 3). In marked contrast, no infiltrating leukocytes were detected in retinas from CD40−/− mice. In addition to the presence of leukocytes, retinas from B6 mice collected 2 days after I/R revealed a marked decrease in the number of ganglion cells (average of 63% decrease; n = 3; p = 0.01; Fig. 1, A and B). In contrast, retinas from CD40−/− mice subjected to I/R revealed a modest and statistically nonsignificant decrease in the number of ganglion cells (average of 26% decrease; n = 3, p > 0.05; Fig. 1, A and B).

Capillary degeneration is an important feature of ishemic retinopathies including those caused by I/R and diabetes. Indeed, retinas from B6 mice revealed a marked increase in the number of degenerate (acellular) capillaries at 8 days post-I/R (n = 5; p < 0.001; Fig. 1C). CD40−/− mice were protected from the capillary degeneration induced by I/R given that there was only a modest increase in the number of acellular capillaries that was statistically nonsignificant (n = 4; p > 0.05). Taken together, these results...
indicate that CD40 is an important mediator of retinal inflammation and promotes neurovascular degeneration.

**CD40 controls NOS2 and COX-2 expression in the ischemic retina**

We conducted experiments to identify mechanisms by which CD40 mediates retinal injury. NOS2 plays an important role in neurovascular degeneration in retinopathies including those mediated by acute ischemia and diabetes (1, 3, 5, 38, 39). In addition, COX-2 promotes neuronal injury after ischemia (40). Thus, we examined whether CD40 controls the expression of NOS2 and COX-2 after I/R of the retina. I/R causes a marked up-regulation of NOS2 and COX-2 mRNA in the retinas of B6 mice (Fig. 2). In contrast, CD40−/− mice were protected from I/R-induced NOS2/COX-2 up-regulation (n = 3; p < 0.02). These results indicate that CD40 controls in vivo cellular responses linked to neural degeneration and inflammation.

Immunohistochemistry was performed to identify cells that express NOS2 and COX-2 in the early stages of retinal ischemia. While NOS2 was not detected in normal mouse retina, some cells in the ganglion cell layer and inner nuclear layer were weakly positive for COX-2 (Fig. 3) (41). Infiltrating leukocytes that were intensely positive for NOS2 and COX-2 were detected in the vitreous and retinas from B6 mice subjected to I/R (Fig. 3). Thus, CD40 regulates the expression of NOS2 and COX-2 in part by promoting recruitment of leukocytes that express these molecules.

**CD40 mediates up-regulation of KC/CXCL1 and ICAM-1 in the ischemic retina**

The retina is infiltrated by polymorphonuclear leukocytes in the early stages after ischemia (39). We hypothesized that CD40 mediates up-regulation of KC/CXCL1 in ischemic retinas because KC/CXCL1 can be produced by nonhemopoietic cells and mediates recruitment of polymorphonuclear leukocytes (42). Using real-time PCR, we detected a marked up-regulation of KC/CXCL1 mRNA levels in retinas from B6 mice subjected to I/R (Fig. 4). Such up-regulation was abrogated in ischemic retinas from CD40−/− mice (n = 4; p = 0.01).

Next, we examined whether CD40 promotes up-regulation of the adhesion molecule ICAM-1 after I/R. ICAM-1 mRNA levels were increased in retinas from B6 mice subjected to I/R (Fig. 4). Such up-regulation was significantly inhibited in ischemic retinas from CD40−/− mice (n = 4; p = 0.03). Thus, CD40 regulates retinal expression of KC/CXCL1 and ICAM-1, events known to mediate leukocyte recruitment.

**CD40 induces ICAM-1 up-regulation as well as KC/CXCL1 production by retinal cells**

Endothelial and Muller cells are major components of the blood-retinal barrier (43). Thus, we reasoned that these cells might be involved in CD40-dependent expression of KC/CXCL1 and ICAM-1. To begin to test this hypothesis we determined whether these cells express CD40. Suspensions of primary mouse retinal cells were stained with Abs against CD154, vimentin (markers for endothelial and Muller cells, respectively), CD40, or control Abs. Flow cytometry analysis of gated CD105⁺ and vimentin⁺ retinal cells revealed expression of CD40 (Fig. 5). In contrast, photoreceptors (the major subset of retinal cells) identified by intracellular staining with an anti-rhodopsin mAb, did not express CD40 (Fig. 5C). We also used flow cytometry to examine whether the phenotypic composition of retinal cells as it relates to endothelial and Muller cells were different in B6 compared with CD40−/− mice. The
Expression of CD40 in the retina is required for development of ischemic retinopathy

We conducted experiments to determine whether development of ischemic retinopathy required tissue expression of CD40. Bone marrow chimeras were generated using CD40+/+ (B6) and CD40−/− mice. B6→B6 and CD40−/−→CD40−/− transplants were generated as controls, and they behaved like nontransplanted B6 and CD40−/− animals, respectively. One eye per mouse was subjected to I/R at 4 wk posttransplant. As shown in Fig. 8, KO→B6 and B6→B6 mice exhibited leukocytic infiltrates and prominent loss of ganglion cells after retinal ischemia. In contrast, these findings were ameliorated in CD40−/−→KO mice (p < 0.05). In addition, although ICAM-1 and KC/CXCL1 mRNA were markedly up-regulated in KO→B6 mice, expression of these genes was blunted in B6→KO mice. Taken together, these studies indicate that whereas the origin of leukocytes (CD40+/+ or CD40−/− animals) has little effect on the development of retinopathy, tissue expression of CD40 in the retina is pivotal for development of ischemic retinopathy.

Discussion

A complex mixture of various inflammatory mediators is increasingly recognized to be important for the development of various retinopathies. Our objective was to characterize the molecular mechanisms responsible for the retinal inflammatory response. We used CD40−/− mice to investigate the role of this molecule in the development of ischemic retinopathy. We report that CD40 is critical for the development of ischemic retinopathy because CD40−/− mice subjected to I/R did not develop inflammation and were protected from neurovascular degeneration. CD40 controls two key mediators linked to neurovascular degeneration: NOS2 and COX-2, at least in part, by promoting retinal infiltration with leukocytes that express these molecules. Our studies also revealed that CD40 is expressed not only on retinal endothelial cells but also on Muller cells. CD40 is an inducer of KC/CXCL1 given that retininas from CD40−/− mice did not up-regulate KC/CXCL1 mRNA and CD40 stimulation of retinal endothelial cells triggered KC/CXCL1 production. We observed that CD40 controls ICAM-1 up-regulation in ischemic retinas and causes up-regulation of this adhesion molecule on retinal endothelial and Muller cells. Finally, bone marrow transplant experiments revealed that

percentages of retinal cells that were CD105+ and vimentin+ were 0.9 and 36%, respectively, in B6 mice (n = 4). Similar percentages were observed in CD40−/− mice (0.8 and 35%; n = 2). Thus, it appears unlikely that the different responses to retinal ischemia observed in B6 compared with CD40−/− mice were explained by changes in retinal endothelial/Muller cell development in CD40−/− animals.

We used lines of mouse retinal endothelial cells and retinal glia with Muller cell characteristics to determine whether CD40 stimulation up-regulates ICAM-1. Incubation of these cells with mouse CD154 significantly increased expression of ICAM-1 on CD40+ cells (Fig. 6; p < 0.001). Next, we examined the effects of CD40 stimulation on KC/CXCL1 secretion. IL-1 is an important proinflammatory cytokine induced after ischemia that regulates chemokine production and mediates tissue injury (44, 45). We explored whether CD40 and IL-1 cooperate to induce KC/CXCL1 production. Low concentrations of IL-1β (25 pg/ml) were used for these experiments. Compared with retinal endothelial cells incubated with CD154 or IL-1β alone, coinubcation with CD154 plus IL-1β resulted in an increase in secretion of KC/CXCL1 (Fig. 7; p < 0.01). Taken together, CD40 promotes retinal endothelial and Muller cell expression of molecules key for leukocyte recruitment.

Discussion

A complex mixture of various inflammatory mediators is increasingly recognized to be important for the development of various retinopathies. Our objective was to characterize the molecular mechanisms responsible for the retinal inflammatory response. We used CD40−/− mice to investigate the role of this molecule in the development of ischemic retinopathy. We report that CD40 is critical for the development of ischemic retinopathy because CD40−/− mice subjected to I/R did not develop inflammation and were protected from neurovascular degeneration. CD40 controls two key mediators linked to neurovascular degeneration: NOS2 and COX-2, at least in part, by promoting retinal infiltration with leukocytes that express these molecules. Our studies also revealed that CD40 is expressed not only on retinal endothelial cells but also on Muller cells. CD40 is an inducer of KC/CXCL1 given that retininas from CD40−/− mice did not up-regulate KC/CXCL1 mRNA and CD40 stimulation of retinal endothelial cells triggered KC/CXCL1 production. We observed that CD40 controls ICAM-1 up-regulation in ischemic retinas and causes up-regulation of this adhesion molecule on retinal endothelial and Muller cells. Finally, bone marrow transplant experiments revealed that
tissue expression of CD40 in the retina but not on peripheral blood leukocytes modulated proinflammatory responses and neuronal loss. Taken together, these findings are compatible with a model in which ischemia-induced activation of CD40 expressed on retinal endothelial and Muller cells would cause KC/CXCL1 production and ICAM-1 up-regulation, leading to recruitment of leukocytes that express NOS2 and COX-2, mediators linked to neurovascular degeneration in the retina.

Retinopathy induced by I/R reproduces important aspects of diabetic retinopathy such as neurodegeneration and capillary degeneration secondary to inflammation (1). Moreover, like diabetic retinopathy, retinopathy induced by I/R causes up-regulation of NOS2, COX-2, and IL-1 (39, 44, 46), and therapeutic approaches that inhibit development of diabetic retinopathy in animals (blockade or inhibition of the expression of NOS2, TNF-α, and IL-1) likewise inhibit the retinal pathology after I/R (7, 39, 44, 46, 47). These overlapping features between diabetic and I/R-induced retinopathies raise the possibility that CD40 is a mediator also of diabetic retinopathy. Relevant to this possibility is the evidence that points to activation of the CD40-CD154 pathway in diabetic retinopathy, because CD154 expression is increased in patients with this disease (48, 49).

Muller cells are the principal glia in the retina. They link neurons with blood vessels anatomically and physiologically and play a major homeostatic role in the retina (50). Muller cells acquire expression of proinflammatory genes during retinopathies (51, 52). Thus, these cells likely contribute to tissue injury in retinopathies (51). Our finding that Muller cells express CD40 and that this receptor activates adhesion molecule up-regulation in these cells suggests that CD40 may be an important activator of retinal inflammation and injury promoted by Muller cells.

Leukocyte recruitment is an important event in the pathogenesis of various retinopathies. For example, leukocyte accumulation in the retina leads to retinal injury after I/R (8), and leukostasis in diabetic retinopathy is associated with breakdown of the blood-retinal barrier and endothelial cell death (9, 53). One of the most striking effects of the absence of CD40 is the impaired leukocyte recruitment to the ischemic retina. We hypothesized that CD40 up-regulates KC/CXCL1 because this chemokine is known to induce recruitment of polymorphonuclear leukocytes and to be produced by nonhemopoietic cells (42) and thus might be secreted by resident nonhemopoietic retinal cells in response to CD40 signaling. Indeed, up-regulation of KC/CXCL1 in ischemic retinas was ablated in CD40−/− mice, and CD40 stimulation of retinal endothelial cells caused KC/CXCL1 secretion. In contrast to our studies, CD40 stimulation was not found to trigger KC/CXCL1 production by B cells (54). These different responses are likely explained by the fact that the effects of CD40 stimulation can be cell specific. The relevance of KC/CXCL1 up-regulation is supported by the demonstration that administration of neutralizing Ab against KC/CXCL1 Ab blocks polymorphonuclear leukocyte recruitment to ischemic organs (55). Although KC/CXCL1 is a known recruiter of polymorphonuclear leukocytes, this chemokine has been reported to also mediate arrest of monocytes (56). This raises the possibility that KC/CXCL1 may also be responsible for monocyte recruitment to ischemic retina that follows polymorphonuclear leukocyte influx (39). CD40 can induce expression of MCP-1 (14). However, CD40 does not appear to be a major regulator of MCP-1 expression in ischemic retinas. Although retinal ischemia causes up-regulation of MCP-1 (57), ischemic retinas of CD40−/− mice exhibited diminished mRNA levels of MCP-1 that did not reach statistical significance (J.-A. C. Portillo, G. Okenka, T. Kern, and C. Subauste, unpublished observations).

The rapid expression of IL-1 after ischemia and the role of this cytokine in chemokine secretion and tissue injury (44, 45, 58) provided rationale for exploring whether CD40 and IL-1 cooperate to produce KC/CXCL1 by retinal cells. We report that CD40 stimulation of retinal endothelial cells in the presence of IL-1β results in a marked increase in production of KC/CXCL1. This effect may be explained by synergy of signaling cascades downstream of CD40 and IL-1 receptors as well as up-regulation of CD40 caused by IL-1β. Relevant to our findings is the report that CD40 and IL-1β cooperate to activate renal tubular epithelial cells (59). Although these findings support CD40 and IL-1β act in concert to promote inflammation, our in vivo results with CD40−/− mice indicate that absence of CD40 is sufficient to abrogate a variety of inflammatory responses in the ischemic retina.

CD40 also mediates ICAM-1 up-regulation in the ischemic retina as well as on retinal endothelial and Muller cells. Our studies suggest that although CD40 partially mediates ICAM-1 up-regulation in ischemic retinas, CD40 appears to be more important for KC/CXCL1 up-regulation. Relevant to these findings is the evidence that leukocytes can be recruited to tissues by CXC chemokines in an apparent ICAM-1-independent manner (60).

Neurovascular degeneration is an important pathological feature of various retinopathies, including those caused by diabetes and acute ischemia (1). The mechanisms that trigger retinal neurovascular degeneration are not fully understood. This work identified CD40 as a mediator of this process. CD40 promoted up-regulation of NOS2 and COX-2, molecules linked to neuronal and endothelial cell death. NO promotes cell death at least in part through formation of peroxynitrite (61). NOS2 is up-regulated not only in acute retinal ischemia (1) but also in diabetic retinopathy (5, 62). Induction of NOS2 is relevant because administration of a NOS2 inhibitor partially protects against retinal thinning after ischemia and diabetic retinopathy (3, 4), and NOS2−/− mice are resistant to diabetic retinopathy (5). Metabolites downstream of COX-2 likely explain the neurotoxicity after ischemia (63). COX-2 is up-regulated in I/R-induced retinopathy, ischemic proliferative retinopathy, and diabetic retinopathy (1, 6, 62, 64, 65). Moreover, COX inhibitors prevent the development of retinal acellular capillaries in diabetes (7, 62, 65).

Leukocytes recruited to the ischemic retina express NOS2 and COX-2. Thus, the role of CD40 in the recruitment of leukocytes explains in part how this molecule regulates NOS2 and COX-2 expression. However, it is likely that there are additional mechanisms by which CD40 promotes retinal expression of NOS2 and COX-2. Whereas at 1 day after retinal ischemia NOS2 is expressed in leukocytes, resident retinal cells acquire expression of NOS2 several days after ischemia (39). Similarly, studies in patients and animals suggest that Muller cells express NOS2 in ischemic and diabetic retinopathies (66, 67). The fact that Muller cells express CD40 and that CD40 stimulation can induce NOS2 and COX-2 expression (16, 19) raises the possibility that direct engagement of CD40 in resident retinal cells may also result in NOS2 and/or COX-2 expression. These possibilities are currently being studied in our laboratories.

Inflammation induced by ischemia is a contributor to pathology not only in retinopathies but also in other conditions such as stroke and organ injury following transplant and cardiopulmonary bypass. Moreover, CD40 promotes tissue injury in stroke, pulmonary, and liver I/R (68–70). Thus, our studies may be relevant to conditions other than retinopathies and expand the knowledge of the role of CD40 in ischemic injury by demonstrating that CD40 controls in vivo expression of NOS2, COX-2, and KC/CXCL1.

The studies presented here may have therapeutic implications for the management of retinopathies and ischemic injury.
acts as an upstream activator of various molecules that induce inflammation and cell death in the retina, and the lack of CD40 inhibits degeneration of neuronal and vascular cells. Studies in animals indicate that CD40 is a molecular target for management of various inflammatory and neurodegenerative disorders (22–24). Clinical trials related to autoimmunity have focused on the administration of blocking anti-CD154 mAb. This approach appeared to be effective but was accompanied by side effects that were likely not directly related to CD40 blockade (71, 72). Thus, new strategies to block CD40 signaling may prove useful for treatment of the various CD40-driven disorders. Such an approach may also result in neuroprotection, a major goal in the therapy of retinopathies that unfortunately has not been achieved with currently available therapeutic strategies.

Acknowledgments

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