CEACAM1 Confers Resistance Toward Oxygen-Induced Vessel Damage in a Mouse Model of Retinopathy of Prematurity

Peter Ludewig, Kai Flachsbarth, Claudia Wegscheid, Gisa Tiegs, Gisbert Richard, Christoph Wagener, Udo Bartsch, and Andrea Kristina Horst

1Institute of Clinical Chemistry, Center for Diagnostics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
2Department of Ophthalmology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
3Institute of Experimental Immunology and Hepatology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence: Andrea Kristina Horst, Institute of Clinical Chemistry, Center for Diagnostics, University Medical Center Hamburg-Eppendorf, CAMPUS Research Building N27, Martinistraße 52, D-20246 Hamburg, Germany; ahorst@uke.de.

Udo Bartsch, Experimental Ophthalmology, Department of Ophthalmology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, D-20246 Hamburg, Germany; u.bartsch@uke.de.

AKH and UB are joint senior authors.

Submitted: October 08, 2013
Accepted: October 29, 2014


PURPOSE. To determine a functional role for the carcinoembryonic antigen-related cell-adhesion molecule 1 (CEACAM1) in retinal neovascularization in a mouse model of oxygen-induced retinopathy (OIR).

METHODS. In a 21/75/21% OIR mouse model, retinal neovascularization was compared in wild-type and CEACAM1-deficient mice. Animals were housed under normoxic conditions until postnatal day 7, followed by exposure to 75% oxygen for 5 days, and further housing under normoxic conditions. Retinal vascular anatomy, vaso-obliteration, neovascularization, and tuft formation were characterized and quantified in retinal flat-mounts from untreated mice and from experimental mice during and at different time points after exposure to high oxygen levels. The vascular network was stained with fluorescently labeled isoelectin B4.

RESULTS. Mice deficient in CEACAM1 did not present any apparent abnormalities in their postnatal retinal vascular development under normoxic housing conditions. However, after hyperoxia and under relative hypoxic conditions, retinal neovascularization and tuft formation were aggravated in the mutant. Congruently, revascularization and vessel maturation were delayed in CEACAM1-deficient mice whereas in wild-type mice, tuft regression and vascular remodeling occurred efficiently after exposure to high oxygen levels.

CONCLUSIONS. Our report describes a functional role for CEACAM1 in retinal neovascularization in a mouse model of OIR. This is the first study demonstrating that CEACAM1 enhances vascular remodeling and tuft regression by increasing endothelial resistance to alterations in oxygen tension, thus accelerating vascular recovery after systemic hypoxia.

Keywords: neovascularization, retinal ischemia, vascular remodeling, retinal vasculature, retinopathy of prematurity

Retinal neovascularization is a leading cause of blindness in developed countries. The vitreous is physiologically avascular but becomes vascularized in diseases such as (proliferative) diabetic retinopathy, retinopathy of prematurity, or retinal vein occlusion. The underlying cause for this pathological vessel growth from the retina into the vitreous often is ischemia or inflammation that results from local or systemic hypoxia or vascular dysfunction. Hypoxia and inflammation are key regulators of angiogenesis via hypoxia-inducible factor-1 alpha (HIF-1α)– and HIF-2α–mediated upregulation of vasoactive proteins, such as VEGF-A, glucose transporters, erythropoietin, or heme oxygenase. Among these, VEGF-A is the most prominent target for antiangiogenic therapies. The anti-VEGF-A antibody bevacizumab (Avastin) or its Fab fragment, ranibizumab (Lucentis), induce vascular regression starting within the first day of application. However, it has been shown that the duration of the therapeutic effects is limited to 2 to 11 weeks, depending on the efficacy of delivery. Furthermore, antiangiogenic therapies have been demonstrated to cause undesired side effects, especially in the treatment of tumors, such as enhanced vascular pruning and reduction in perfusion, which is a drawback for therapeutics delivery.

A common goal in antiangiogenic therapies is thus to stabilize the delicate balance between the upkeep of therapeutic efficacy and prevention of inappropriate vessel regression. Mancuso et al. have demonstrated that VEGF-targeting therapeutics leave the perivascular sleeves of collagenous basement membranes and pericytes intact, providing a scaffold for revascularization. Hence, interruption of antiangiogenic treatment leads to rapid vascular regrowth, and consequently, antiangiogenic therapies are commonly scheduled metronomically. It is therefore of importance to identify novel angiogenic molecules that may harbor future antiangiogenic or anti-inflammatory therapeutic options and that may support and further improve existing therapies.

The carcinoembryonic antigen-related cell-adhesion molecule 1 (CEACAM1) is expressed on epithelia, leukocytes, and endothelia. The CEACAM1 was identified as a marker of newly formed vessels and as a proangiogenic factor in human placenta and a variety of tumors. Furthermore, we and others have demonstrated proangiogenic properties of CEACAM1 in inflammation and tissue remodeling in various other...
context. In a mouse model of chronic cutaneous infection, for instance, CEACAM1 expression on myeloid cells and endothelia supported resolution of inflammation and enhanced lymph-hematangiogenesis in the granulation tissue. In a mouse model of mammary carcinoma, peritumoral vascular endothelial CEACAM1 expression aggravated tumor angiogenesis, and in a mouse model of melanoma, tumor angiogenesis was regulated in a CEACAM1-dependent manner by inhibiting secretion of the angiogenic factor prokineticin-2/Bombina variegata 8 kDa. In addition, CEACAM1 has been identified as a negative modulator of vascular permeability via regulation of endothelial nitric oxide synthetase (eNOS) activation, and as a vasoprotective factor in the myocard after hypoxic preconditioning.7,25,26 A recent report also addressed the vasoprotective influence of CEACAM1 on aortic endothelia, detailing the effects of CEACAM1-mediated effects on endothelial insulin metabolism, and eNOS and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity.31 However, it is not yet known whether CEACAM1 plays a role in physiological vascularization during postnatal development or in retinal neovascularization. In this study, we therefore investigated whether endothelial expression of CEACAM1 is of relevance for retinal vascularization during normal development. Furthermore, we evaluated the role of CEACAM1 in retinal neovascularization in a mouse model of oxygen-induced retinopathy (OIR).

Materials and Methods

Animals

Mice deficient in CEACAM1 were maintained on a C57BL/6J genetic background (B6.Ceacam1<sup>−/−</sup> mice) and genotyped as described elsewhere.32 Age-matched wild-type mice on the same genetic background (B6.WT mice) served as a control. Mice were housed in a specific pathogen-free (SPF)-certified facility and kept under a standard 12-hour light/dark cycle. Animals were fed ad libitum and all experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the Federation for Laboratory Animal Science Associations (FELASA) guidelines, and the German Law for the Protection of Animals. All animal experiments were approved by the animal committee of the state of Hamburg; reference no.: G09/018 and G14/005.

Mouse Model of OIR

Oxygen-induced retinopathy was induced in young postnatal mice as described in Smith et al.33 In brief, mice were exposed to 75% oxygen between postnatal day 7 (P7) and P12 in a Biospherix incubator (Biospherix Ltd., Lacona, NY, USA). Oxygen saturation was controlled using a ProOx oxygen probe (Biospherix Ltd.). On P12, relative hypoxia was induced by transferring the mice to standard housing conditions. Animals were analyzed on P7, P12, P17, and P19. Because fostering and vitamin D influence outcome after OIR, we only included mice with a minimum weight of 4.5 g (P12), 5.5 g (P17), and 6.5 g (P19) in our experiments.34

Analyses of Retinal Flat-Mounts

To analyze retinal flat-mounts, animals were killed and eyes were quickly removed and immersion-fixed in 4% paraformaldehyde (PFA) for 15 minutes at room temperature. Subsequently, retinal flat-mounts were prepared, post-fixed in 4% PFA for 45 minutes, and permeabilized in ice-cold 70% ethanol for 20 minutes at −20°C. Unspecific binding sites were blocked in PBLEC blocking buffer (PBS, pH 6.8, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1% Triton X-100), 5% bovine serum albumin (BSA), followed by three washes in PBLEC buffer without BSA. Fluorescent labeling and immunohistochemistry were performed with minor modifications as described.35 In brief, retinas were incubated with primary antibodies or isoelectric B<sub>I</sub> (IB<sub>I</sub>) for 48 hours at 4°C in PBLEC buffer containing 0.5% BSA, followed by three extensive washes with PBLEC, and 24 hours of incubation with appropriately labeled secondary antibodies or fluorophore-conjugated streptavidin (see below). Retinal angiogenesis was analyzed after staining with either Alexa-488-conjugated or Cy3-conjugated IB<sub>I</sub>, or biotinylated IB<sub>I</sub> (Invitrogen, Molecular Probes, Carlsbad, CA, USA), as described.35 For CEACAM1 labeling in cross sections of intact bulbi, the eyes were processed for standard immunohistochemistry as described.29 To label CEACAM1, antiserum P126 was used, followed by biotinylated anti-rabbit-antibodies cocktail (DAKO, Glostrup, Denmark) and alkaline phosphatase Vecta-ABC-Kit (Vector Laboratories, Burlingame, CA, USA). Images were taken with a Zeiss Axioskop 2 plus microscope (Carl Zeiss, Jena, Germany) or a Leica DM5000B fluorescence microscope (Leica, Wetzlar, Germany) equipped with a Leica DFC 360 FX camera using HCX PL FLUOTAR 10_/0.3, PLAN APO20_/0.9, HCX PLAPO 40_/0.75, and HCX PLAPO 100_/1.40.7 lenses. Images were processed using Leica LAF software (Leica) and Adobe Photoshop (Adobe Systems, Inc., San Jose, CA, USA).

Quantification of Vascularization, Vaso-Obliteration, and Tuft Formation

For image acquisition of neovascularized retinal areas, the inner limiting membrane was focused to distinguish supranuclear tufts from the underlying vascular network. Retinal flat-mounts were photographed and images were assembled using Adobe Photoshop CS3 software (Adobe Systems, Inc.). Quantification of tuft formation and vaso-obliteration was performed as described elsewhere.29,35

Statistical Analyses

Statistical analyses were performed using GraphPad Prism Software, Version 4.0 (GraphPad Prism Software, Inc., La Jolla, CA, USA). Data sets were analyzed for Gaussian distribution with the F normality test and compared using the Student’s t-test or the Mann-Whitney U test.

Results

Loss of CEACAM1 Does Not Affect Physiological Retinal Vascularization

Before we evaluated the role of CEACAM1 in retinal neovascularization in a mouse model of OIR, we compared retinal vascularization under normoxic conditions in wild-type (B6.WT) and CEACAM1-deficient (B6.Ceacam1<sup>−/−</sup>) mice to evaluate possible phenotypic effects of CEACAM1 deficiency on physiological retinal vascularization. Retinal flat-mounts were prepared from 7- and 12-day-old and 12-week-old untreated B6.WT and B6.Ceacam1<sup>−/−</sup> mice, and stained with B<sub>I</sub>. To monitor postnatal primary vessel plexus regression and arterial versus venous specification and differentiation, we measured the length of the primary plexus in B6.WT and B6.Ceacam1<sup>−/−</sup> mice (Figs. 1A, 1B), as well as the diameters of retinal arteries and veins (Figs. 1C, 1D). There were no significant differences in postnatal vascular development between genotypes. To monitor differences in angiogenic endothelial sprouting capacities between B6.WT and
B6.Ceacam1\(^{-/-}\) mice, we additionally assessed tip cell length (Figs. 1E, 1F), and again observed no significant differences between both genotypes. To follow up on primary plexus regression, we determined the areas of the superficial residual primary vascular plexus of 12-day-old B6.WT and B6.Ceacam1\(^{-/-}\) mice as a measure for postnatal vessel maturation (Figs. 2A–C). As depicted in Figures 2A through 2C, areas of the residual primary plexus of the total retinal area were not significantly different between both genotypes (summarized in Fig. 2C). To further characterize retinal vascular network development and remodeling, we additionally analyzed the complexity of the retinal vascular networks in the superficial, intermediate, and deep retinal layers in 12-week-old mice to monitor possible effects of CEACAM1 on arterial and venous differentiation and overall branching of the vasculature (Figs. 2D–F). The quantification procedure used for the evaluation of network branching and complexity is detailed in Refs. 35 and 36. Subsequently, the total length of the network paths was calculated, and presented as in Figure 2F. As summarized in Figures 1 and 2, there were no differences between B6.WT and B6.Ceacam1\(^{-/-}\) mice regarding regression of the primary plexus (Figs. 1A, 1B, 2A–C), indicative of normal vascular network maturation in the absence of CEACAM1. In both genotypes, the vasculature covered the entire area of the retina, and area fractions of the superficial residual plexus network were 5.56% ± 0.59% of the total retinal area in B6.WT and 6.50% ± 0.76% in B6.Ceacam1\(^{-/-}\) mice. Similarly, the retinal vascularization and network complexity, as well as the arterial and venous branching under physiological conditions in 12-week-old animals did not reveal any significant differences between the genotypes (Figs. 2D–F). In the three retinal layers, vascular network densities and arborization were comparable between B6.WT and B6.Ceacam1\(^{-/-}\) mice (Fig. 2D, for B6.WT mice: superficial layer [sum of total vessel length in millimeters per area: 24.54 ± 1.65 mm/mm\(^2\)], intermediate layer [32.21 ± 2.87 mm/mm\(^2\)], and deep layer [40.62 ± 3.0 mm/mm\(^2\)]; Fig. 2E, for B6.Ceacam1\(^{-/-}\) mice: superficial layer [22.39 ± 1.96 mm/mm\(^2\)], intermediate layer [32.07 ± 2.013 mm/mm\(^2\)], and deep layer [41.21 ± 1.28 mm/mm\(^2\)]).

Loss of CEACAM1 Leads to Increased Vaso-Obliteration in Early Phases of OIR

It has been reported previously that once Ceacam1\(^{-/-}\) mice are challenged by tumor growth, vascular occlusion, wound healing, or altered endothelial metabolism in atherosclerosis, vascular endothelial CEACAM1 expression is an effective regulator of vessel remodeling and angiogenesis.\(^{25,26,28,31,32}\) However, it has not been demonstrated whether hyperoxia affects the vasculature in B6.WT and B6.Ceacam1\(^{-/-}\) mice differently. To assess vaso-obliteration, neovascularization, and vessel remodeling in the OIR model, animals were analyzed on P8, P12, P17, and P19. Animals were evaluated at these specific ages to perform a detailed comparison between both
genotypes with regard to initial vessel damage after 1 day of exposure to high oxygen levels at P8, robustness and development of the vasculature after hyperoxia for 5 days and return to normoxia at P12, the extent of neovascularization after return to normoxia at P17, and the extent of vascular remodeling at P19. As shown in the Supplemental Figure S1, the initial oxygen-induced vaso-obliteration on P8 was slightly but significantly increased in B6.Ceacam1<sup>−/−</sup> mice when compared with WT controls. This finding indicates that B6.Ceacam1<sup>−/−</sup> mice have a higher inherent susceptibility toward oxygen-induced vessel damage than CEACAM1-competent mice. To corroborate these findings, we additionally analyzed the retinal vasculature after 5 days of hyperoxia at P12 (Fig. 3). This analysis revealed that persistence of high oxygen levels during development of the retinal vasculature leaves retinas from B6.Ceacam1<sup>−/−</sup> mice with a significantly larger central obliteration (central obliteration: 31.56% ± 1.48% of the total retinal area; Figs. 3C–E) compared with retinas from B6.WT mice (central obliteration: 22.10% ± 1.38% of the total retinal area; Figs. 3A, 3B, 3E).

In addition to central hypoperfusion, we also assessed the degree of retinal vessel maturation after hyperoxic housing conditions (Fig. 4). As demonstrated in Figure 4, areas of the residual superficial vessel network were significantly smaller in B6.WT (residual plexus area of the entire retinal area: 5.56% ± 0.59%; Figs. 4A, 4B, 4E) than in B6.Ceacam1<sup>−/−</sup> mice (residual plexus area of entire retinal area: 16.18% ± 1.25%; Figs. 4C–E). Taken together, these findings suggest that CEACAM1-deficient mice exhibit increased dependence on oxygen-sensitive growth factors, and/or lower tolerance toward oxidative tissue damage when compared with CEACAM1-competent mice.

**Hypoperfusion and Neovascularization in Retinal Ischemia Is Attenuated by CEACAM1**

To further characterize vascular remodeling in response to hyperoxia, mice were kept under normoxic conditions for 5 or 7 days after exposure to 75% oxygen, and retinas were analyzed for the extent of vaso-obliteration, neovascularization, and tuft formation. Hypoxia as a result of oxygen deprivation or changes in metabolism potentiates the angiogenic response (reviewed in Ref. 37). First evidence that CEACAM1 expression is modulated by hypoxia was provided by the observation that CEACAM1 is strongly upregulated during hypoxic preconditioning in the myocard. Later, we demonstrated that vascular CEACAM1 expression enhanced angiogenesis in hypoxic
calves after femoral artery occlusion. 27 To assess whether CEACAM1 also plays a regulatory role in retinal neovascularization, we analyzed CEACAM1 expression in retinas from B6.WT and B6.Ceacam1<sup>−/-</sup> mice at P17, an age when neovascularization peaks in this mouse model of OIR. As shown in Figures 5A and 5C, CEACAM1 was expressed on newly formed vessels protruding into the vitreous. Tufts in B6.Ceacam1<sup>−/-</sup> mice were negative for CEACAM1, as expected (Fig. 5B).

Because the vascular expression of CEACAM1 suggests a functional role of this protein in retinal neovascularization, we assessed hypoperfusion and neovascularization, evident by tuft formation, at the turning point between the proliferative and regressive phase of OIR at P17. We observed that in B6.Ceacam1<sup>−/-</sup> mice, central vaso-obliteration persisted and was significantly more pronounced when compared with B6.WT mice (Figs. 6A–D). In B6.WT mice, the avascular areas covered approximately 12.5% ± 1.0% of the retinal surface compared with 18.4% ± 0.8% in B6.Ceacam1<sup>−/-</sup> mice (Fig. 6E). In addition, pathological vessel outgrowth was significantly increased in B6.Ceacam1<sup>−/-</sup> mice (tufts covered 5.9% ± 0.3% of the entire retinal area; Fig. 6F) when compared with controls (tufts covered 3.3% ± 0.4% of the entire retinal area; Fig. 6F). Of note, the difference in central vaso-obliteration and tuft formation between genotypes further increased with increasing age of the animals. At P19, the area of central vaso-obliteration was 6.6% ± 0.7% of the total retinal area in B6.WT mice (Figs. 7A, 7B, 7E) as opposed to 16% ± 1% in B6.Ceacam1<sup>−/-</sup> mice (Figs. 7C, 7D, 7E). Furthermore, tufts covered 2.0% ± 0.3% of the total retinal area in 19-day-old B6.WT mice (Figs. 7A, 7B, F) as compared with 5.1% ± 0.4% in age-matched B6.Ceacam1<sup>−/-</sup> mice (Figs. 7C, 7D, 7F).
Remodeling of the Retinal Vasculature Is Enhanced by CEACAM1 in the Proliferative Phase of OIR

The marked differences in vaso-obliteration and neovascularization between both genotypes could either result from the higher initial obliteration observed in 12-day-old B6.Ceacam1−/− mice, or from a perturbation of vessel sprouting, endothelial proliferation, and/or vessel remodeling in B6.Ceacam1−/− mice. To discriminate between these possibilities, we quantified revascularization and tuft regression over time and compared the rate of vessel growth in both genotypes between P12 and P19.

Although the initial obliterated areas were significantly larger in B6.Ceacam1−/− mice (cf. Supplemental Fig. S1, Figs. 3, 6), B6.WT and B6.Ceacam1−/− mice showed comparable rates of revascularization of the hypoperfused areas between P12 and P17 (compare Fig. 3 and Fig. 6). However, whereas revascularization reached a plateau in B6.Ceacam1−/− mice on P17 and did not increase significantly until P19, revascularization in B6.WT retinas continued between P17 and P19, resulting in a more efficient overall retinal revascularization: between P12 and P19, approximately 19.7% of the retina was revascularized in B6.WT mice, compared with 15.6% in B6.Ceacam1−/− mice (calculated from Figs. 3, 6, 7). Regarding the efficacy of vascular maturation and tuft regression, the neovascularized retinal area was significantly smaller in B6.WT mice than in B6.Ceacam1−/− mice at postnatal day 19 (Fig. 7).

**Figure 4.** Comparison of retinal vessel maturation 5 days after exposure to hyperoxia in B6.WT and B6.Ceacam1−/− mice at P12. (A–D) are representative examples of the retinal vasculature in retinal flat-mounts from B6.WT (A, B) and B6.Ceacam1−/− (C, D) mice 5 days after exposure to high oxygen levels. Areas covered by vessels of the residual superficial plexus are highlighted in yellow. (B) and (D) are higher magnifications of the shaded boxes in (A) and (C), respectively. (E) Areas covered by the superficial primary plexus were measured in B6.WT (n = 11; open squares) and B6.Ceacam1−/− (n = 12; filled squares) mice, and expressed as percentage of the total retinal area. Mean values ± SEM are indicated by horizontal lines. (E) ***P < 0.0001. Scale bars: 1 mm (A–D).
Similar to retinal revascularization, tuft regression occurred more efficiently in CEACAM1-competent animals than in CEACAM1-deficient mice: between P17 and P19 the neovascularized retinal area was reduced by 1.27% in B6.WT mice as opposed to 0.76% in B6.Ceacam1−/− mice (calculated from Figs. 6, 7).

Because aberrant vascular sprouting appeared to affect the angiogenic phenotype in CEACAM1-deficient mice, we additionally studied filopodia formation by retinal endothelial tip cells in both genotypes (Supplemental Fig. S2). Although we occasionally observed rarefaction of tip cells in B6.Ceacam1−/− mice, we did not detect significant differences in the overall length of tip cell filopodia between both genotypes (Supplemental Fig. S2). Hence, we conclude that the delay in retinal revascularization observed in CEACAM1-deficient mice is not related to an impaired angiogenic sprouting of retinal tip cells.

**DISCUSSION**

In human diseases characterized by retinal hypervascularization, such as diabetic retinopathy, AMD, or retinopathy of prematurity, the principal stimulus for abnormal vessel growth is hypoxia. Excessive vascularization of the retina, as well as retinal edema and hemorrhage, cause loss of vision and therapeutic interventions therefore target aberrant retinal vessel growth. So far, VEGF-neutralizing antibodies and panretinal laser coagulation are commonly used to treat excessive vascular sprouting into the vitreous, and to eliminate hypoxic tissue.1 9,11 However, disadvantages of these therapies include an increase in local inflammatory responses leading to exacerbation of vessel growth or fibroblast proliferation, or the need of a constant, metronomic application of therapeutics to maintain treatment efficacy.1 Furthermore, anti-VEGF–targeted therapies have been in the limelight because therapy-induced vessel pruning or rapid decrease of local blood flow results in local hypoxia, thus aggravating the angiogenic response and limiting chemotherapeutics delivery, especially in the context of tumor angiogenesis.12,15,31,38–40 Hence, we sought to identify a functional role for the cellular adhesion molecule CEACAM1 in a mouse model for oxygen-induced retinal neovascularization so as to gauge whether CEACAM1 may hold the potential as an antiangiogenic therapeutic target. A proangiogenic role for CEACAM1 has been demonstrated by us and others before.22,25 Lack of CEACAM1 has been reported to affect vascular integrity and permeability during revascularization of Matrigel implants or tumors, and during edema formation in ischemic stroke.25,27,28,41

In the OIR mouse model evaluated in the present study, we observed that in CEACAM1-deficient mice vaso-oblitertion and tuft formation were significantly increased in early and late phases of retinopathy when compared with WT mice. Furthermore, we observed that retinal revascularization and tuft regression after hyperoxia were significantly delayed in B6.Ceacam1−/− mice when compared with WT mice. Of note, under normoxia, the physiological development of the retinal vasculature or arterial and venous branching were not affected by the systemic loss of CEACAM1.
In both physiological and pathological vascularization of the retina, endothelial filopodia formation and vascular sprouting are essential steps to ensure vessel guidance and functional retinal vascularization.\(^{42,43}\) In spite of the fact that tuft formation and excessive vascular outgrowth were enhanced in CEACAM1-deficient mice, we did not observe significant alterations in filopodia formation in the mutant mice. This observation suggests that endothelial cell guidance and tip cell formation are not affected by the absence of endothelial CEACAM1. In line with this finding, CEACAM1 was not among candidate genes that were significantly upregulated in the tip cell fraction of sprouting vessels in a mouse model of OIR.\(^{44}\) In addition, we did not observe any significant differences in microvascular endothelial cell proliferation between WT and CEACAM1-deficient mice.\(^{41}\)

Besides filopodia formation, stalk cell proliferation is essential for vascular outgrowth. During endothelial sprouting, stalk cells are the principal proliferating cells of the endothelium, and in case their proliferation is delayed or impaired, endothelial sprouting also would be reduced, potentially offering an explanation for the delayed revascularization in OIR in CEACAM1-deficient mice. However, because we found comparable revascularization rates in both genotypes in the proliferative phase of OIR, we assume that stalk cell proliferation was not affected by loss of CEACAM1 in this model and does not contribute to the retardation of vascular regrowth. In a sharp contrast to these observations, we
According to Nouvion et al. and Najjar et al., aberrant observations in early phases of OIR in CEACAM1-deficient mice. This reduced vascular resistance, increased vaso-obliteration is survival in hypoxic, hyperproliferative retinas. As a result of oxygen levels, and probably to a reduction in overall cell CEACAM1-negative vessels toward systemic alterations of vascular growth regulatory proteins, such as HIF-1α or VEGF-A, under oxygen-saturating conditions. Thus, we can speculate that our observations point toward reduced resistance of CEACAM1-negative vessels toward systemic alterations of oxygen levels, and probably to a reduction in overall cell survival in hypoxic, hyperproliferative retinas. As a result of this reduced vascular resistance, increased vaso-obliteration is observed in early phases of OIR in CEACAM1-deficient mice. According to Nouvion et al. and Najjar et al., aberrant activity of eNOS and inadequate production of endothelial damaging NO species could add to lack of endothelial resistance toward oxidative stress. Beauchemin et al. demonstrated that the CEACAM1 isoform with a long cytoplasmic domain containing two immune-receptor tyrosine-based inhibition motifs is crucially involved in limiting the activation of eNOS in newly formed vessels of tumors. In large vessels, on the contrary, oxidative damage as a result of enhanced NADPH oxidase and reduced eNOS activity has been reported in CEACAM1-deficient animals, leading to alterations in endothelial cell oxidative metabolism.

The importance of eNOS expression in vascular closure and recovery in OIR has been demonstrated previously: inadequate eNOS expression led to increased superoxide and NO production, as well as enhanced hypoperfusion and exaggerated angiogenesis and aggravated pathology. Therefore, CEACAM1 may affect eNOS activity in OIR, but the CEACAM1-negative endothelium seems to be more susceptible to oxidative insult, as well. To elucidate additional CEACAM1-mediated effects in the OIR mouse model, we additionally analyzed accessory cells, such as pericytes, microglial cells, and myeloid cells to define their putative function in retinal neovascularization and vessel regression. Our previous studies demonstrated that CEACAM1-expressing endothelial cells are necessary, but not sufficient, for a CEACAM1-dependent effect in vessel remodeling. Pericytes and microglial cells have been described as critical determinants in retinal neovascularization, and myeloid cells have been identified as chaperones and interaction partners for the endothelial vasculature. In the present study, however, we could not detect differences in pericyte coverage of the retinal vasculature between both genotypes, nor could we convey any significant protective effects to the retinal vasculature by intravitreal transplantations of CEACAM1-positive microglial precursor cells into B6.Ceacam1−/− mice after induction of OIR.

Taken together, we demonstrate that vascular endothelial CEACAM1 expression enhances endothelial and probably also myeloid resistance toward oxidative stress and hypoxia. Importantly, vessel maturation and vascular recovery were facilitated by endothelial CEACAM1 expression.

In summary, we have demonstrated for the first time that CEACAM1 is an important functional regulator in retinal neovascularization, and limits hypoperfusion and hypoxic or oxidative tissue damage in a mouse model of OIR. Further studies are under way to dissect the endothelial and myeloid contribution to CEACAM1-dependent vessel repair in OIR, and to elucidate which CEACAM1-expressing cell type may render a suitable antiangiogenic therapeutic target.

Acknowledgments

Disclosure: P. Ludewig, None; K. Flachsbart, None; C. Wegscheid, None; G. Tiegs, None; G. Richard, None; C. Wagener, None; U. Bartsch, None; A.K. Horst, None

References

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15. Avery RL. What is the evidence for systemic effects of intravitreal anti-VEGF agents, and should we be concerned? *Br J Ophtalmol.* 2014;98(suppl 1):7–110.


