Endothelial dysfunction in experimental models of preclinical diabetic retinopathy

McWilliams Hughes, J.

Citation for published version (APA):
Chapter 2

Leukostasis and inflammation: Crucial Steps in the Development of Diabetic Retinopathy or Epiphenomenon?

John M. Hughes,
Ingeborg Klaassen,
Cornelis J.F. van Noorden,
Reinier O. Schlingemann

Ocular Angiogenesis Group, Departments of Ophthalmology and Cell Biology and Histology, Academic Medical Center, University of Amsterdam, The Netherlands.

Manuscript in preparation
The role of leukostasis in diabetic retinopathy
Introduction

Over the last ten years, increasing evidence has been found linking leukostasis in the retinal microvasculature of diabetic animals to the development of diabetic-like retinopathy. It has been hypothesized that this increase in static leukocytes is a mild form of inflammation which plays a causal role in the development of vascular abnormalities in the early stages of diabetic retinopathy (DR) and in turn leads to proliferative DR and vision loss in humans. Here, we critically review the current literature regarding leukostasis and assess its pathological consequences in the human diabetic retina. We begin by reviewing the known pathological processes involved in the development of human DR. This is followed by a summary of experimental evidence for the role that leukostasis plays in the development of DR and the mechanisms involved in leukostasis. We then summarize all factors and conditions known to regulate retinal leukostasis. We conclude by discussing these data and synthesizing an answer to the question: Is leukostasis in the diabetic retina a crucial step in the development of DR, or is it merely an epiphenomenon of the diabetic milieu?

Characteristics of Human DR

DR is a leading cause of blindness in working-age individuals in developed countries. The earliest clinically observable changes in the diabetic retina are vascular-related and, as such, DR has traditionally been considered a vascular disease. Pericyte loss, thickening of the vascular lamina basalis (LB), breakdown of the blood-retina barrier (BRB), and acellular capillaries are preclinical phenomena regarded as hallmarks of the onset of DR. As the disease progresses, saccular microaneurysms and hemorrhages appear and increasingly larger areas of non-perfused capillaries become evident. This non-perfusion, in combination with the loss of BRB, facilitates the formation of retinal exudates, cotton-wool spot formation and retinal edema. Intraretinal microvascular anomalies, which consist of dilated, elongated or abnormally tortuous capillaries, are also present. When the areas of non-perfused retina become large enough, neovascularization occurs which leads to vitreal hemorrhaging and/or retinal detachment with subsequent vision loss.

Leukostasis in the diabetic retina

The phenomenon of leukostasis in the vasculature of the diabetic retina was first described by Schröder et al in their histochemical study of retinal whole-mounts obtained from perfusion-fixed diabetic rats, a significant increase in the number of capillary occluding monocytes and granulocytes was observed. These occluding leukocytes also displayed a strong spatial correlation with focal endothelial swelling, capillary loss, and formation of intraretinal microvascular anomalies. Due to the increased numbers of activated leukocytes in diabetes and their ability to cause cell damage by releasing cytotoxic products, it was hypothesized that these occluding leukocytes play a causal
role in the pathogenesis of DR through the induction of direct damage to the endothelium and surrounding tissue.⁴

In vitro studies on isolated retinal microvascular endothelial cells have been performed to further elucidate the specific effects of the hyperglycemic milieu on leukostasis. In one study, bovine retinal endothelial cells (BRECs) were exposed to high concentrations of glucose. This resulted in a dose-dependent increase in neutrophil adhesion. However, the same effect was achieved when the BRECs were incubated with identical concentrations of mannitol. The authors concluded that the observed increase in leukocyte entrapment was due to hyperosmolarity and not the specific effects of excess glucose⁵ A similar experiment performed with human retinal endothelial cells (HRECs) also revealed elevated neutrophil adhesion. High glucose concentrations (46.1 mM) caused adhesion whereas mannitol (30 mM) and L-glucose (30 mM) did not.⁶ This suggests that the increased neutrophil adhesion to HRECs is due to glucose specific reaction specific and not to a non-specific reaction to a hyperosmotic environment.

A technique developed by Nishiwaki et al. enabled the analysis of leukocyte dynamics in the retinal microvasculature in vivo.⁷ Leukocytes were labeled through intravenous injection of the nuclear dye acridine orange and then visualized in the retinal microvasculature using a scanning laser ophthalmoscope. Leukostasis was first shown to be increased in the diabetic retinal microvasculature in vivo using this technique.⁸ Rats with STZ-induced diabetes of 4 weeks duration and spontaneously diabetic Otsuka long-evans Tokushima Fatty (OLTEF) rats, which were hyperglycemic for 6 weeks, were used in the experiment. Leukostasis was increased approximately 2- to 3-fold in both the STZ and OLTEF models. Similar results were obtained using this technique in Zucker diabetic fatty rats,⁹ the spontaneous diabetic Torii (SDT) rat¹⁰ and spontaneous DM type 2 rhesus monkeys.¹¹ In the latter study, retinas with histological evidence of DR and retinas without DR contained similar numbers of static leukocytes. In contrast, the db/db mouse, a model for diabetic dyslipidemia, demonstrated no increase in leukostasis.¹² Unfortunately, the toxic nature of acridine orange prevents the study of leukostasis in the retinal vasculature of diabetic humans. To circumvent this problem, Paques et al. performed a study to validate the use of fluorescein-labeled autologous leukocytes for the examination of retinal circulation in humans.¹³ Two static leukocytes were found in the retina of a single patient with type 1 diabetes whereas leukostasis was not observed in healthy retinas. However, the validity of this technique for studying leukostasis is dubious because labeling with fluorescein leads to the upregulation of adhesion molecules that induce interactions with vascular endothelial cells. Other techniques for the study of leukostasis in human retinal vasculature are not currently available. Therefore, the majority of current data on retinal leukostasis is derived from diabetic rodent in vivo experiments using the acridine orange fluorography and perfusion-fixed flat-mount techniques.
Proposed mechanisms of leukostasis

There are three general mechanisms that have been proposed to lead to increased leukostasis: decreased retinal blood flow or perfusion pressure, narrowing of the capillary lumens, and increased leukocyte-endothelium adhesion. The state of retinal blood flow in diabetes is controversial.\textsuperscript{14,15} Several studies of retinal hemodynamics in diabetic rats have shown that the number of static leukocytes is increased whereas leukocyte passage time through retinal capillaries is similar to that in controls.\textsuperscript{8,9} The latter indicates that decreased retinal blood flow is an unlikely mechanism for increased retinal leukostasis.

Narrowing of retina capillaries has only been observed in diabetic OLETF rats\textsuperscript{16} and primates with VEGF-induced retinopathy.\textsuperscript{17} Moreover, leukocytes isolated from diabetic subjects have decreased deformability.\textsuperscript{18} As the diameter of most leukocytes is roughly twice that of the average retinal capillary, adequate deformability is crucial for leukocyte passage. Capillary lumen size in diabetic retinas shows a significantly larger variance reflecting their more tortuous nature with both widening and narrowing of the capillary lumens.\textsuperscript{10,16,19} Hypertrophy of endothelial cells was shown to cause lumen narrowing in retinal capillaries of primates with VEGF induced retinopathy.\textsuperscript{17} Hypertrophic endothelial cells were also observed in leukocyte-occluded retinal capillaries in STZ-induced diabetic rats, however no difference in mean lumen diameters between diabetic and control rats was measured.\textsuperscript{8} Vascular compression due to retinal edema has been suggested to cause lumen narrowing.\textsuperscript{20} However, this theory is based on an observational study and has not been further studied. Thickening of the LB has also been proposed to lead to capillary narrowing. While a histopathological feature of DR, it has, as yet, not been shown to cause lumen narrowing or leukostasis.\textsuperscript{21} Furthermore, the vascular complications attributed to increased leukostasis have been shown to occur non-uniformly throughout the retina in contrast to LB thickening which occurs more uniformly, making this mechanism less likely.\textsuperscript{22}

Vascular constriction has also been proposed as a mechanism leading to increased leukostasis through capillary narrowing. Levels of the vasoconstrictor endothelin-1 (ET-1) have been found to be increased in diabetic retinal tissue as well as increased expression of endothelin receptors on retinal vascular pericytes.\textsuperscript{23} Additional functional studies have shown that endothelin receptor antagonists reduced the increased leukostasis observed in STZ rat retinas, but this was attributed to decreased VEGF production rather than vasoconstriction.\textsuperscript{24} The extent to which capillary constriction contributes to increased leukostasis in the diabetic retina remains unclear.

Considerably more evidence has been generated implicating a sterile inflammatory process in which increased leukocyte-endothelial adhesion is the major mechanism of increased leukostasis in diabetic retinas.\textsuperscript{1} Leukocytes isolated from diabetic rats and humans have shown increased adhesion to endothelial cells in vitro.\textsuperscript{25,26} Leukocytes from diabetic patients express increased levels of the $\beta_2$-integrins CD11a, CD11b, and CD18, components of two leukocyte adhesion molecules, LFA-1 and Mac-1. These adhesion molecules, in conjunction with their endothelial counterparts ICAM-1 and VCAM-1 play a crucial role in the inflammatory process as they are required for firm
The role of leukostasis in diabetic retinopathy

Leukocytes-endothelial cell adhesion. Antibodies against CD11a, CD11b and CD18 prevented the increased adhesion. CD18 blockade suppressed the increased leukocyte adhesion in retinal capillaries of the diabetic rat in vivo.22 Furthermore, activated lymphocytes that were injected into normal rats were shown to interact with the retinal endothelium, in association with increased ICAM-1 staining of the surrounding endothelium.27 This indicates that activated lymphocytes have the capacity to upregulate endothelial ICAM-1 expression. Administration of anti-ICAM-1 antibodies significantly reduced increased leukostasis in diabetic rats.28,29 Increased leukostasis did not occur in ICAM-1- or CD18-deficient mice after 11 months of STZ induced diabetes or 22 months of galactosemia. Remarkably, leukostasis after 1 week and 11 months of diabetes was comparable in wild-type diabetic mice. The wild-type diabetic mice showed increased numbers of propidium iodide-stained retinal vascular cells indicating an increase in dead or dying cells, which was not the case in the ICAM-1-/- and CD18-/- mice. The deficient mice also displayed reduced DR-associated retinal vascular pathology including blood-retinal barrier breakdown, endothelial cell and pericyte loss, and acellular capillaries. Interestingly, LB thickening was not reduced in the adhesion molecule deficient mice.29

The relevance of ICAM-1 expression in the diabetic human retina is not yet established. Both increased and unaltered ICAM-1 immunohistochemical staining has been reported.30,31 The difference may be explained by the size of the control groups. The former study used a small group of six control retinas, four of which were completely free of ICAM-1 staining, whereas the latter study contained a control group equal in size to the diabetic groups that were studied (n=19). The majority of the control retinas exhibited low to moderate ICAM-1 staining, whereas the diabetic groups showed similar staining. Further supporting this finding are two separate studies in which moderate constitutive endothelial ICAM-1 expression in human retinal capillaries in vivo and in human retinal endothelial cells in vitro has also been reported.32,33 Additionally, the expression of VCAM-1 and the adhesion molecules E-selectin, P-selectin, also important to the inflammatory process through their tethering of leukocytes to the endothelium, was examined immunohistochemically. Interestingly, the expression of these molecules was not observed in the human retinal microvasculature.30,31

Current paradigm of leukocyte-induced vascular pathology leading to DR

In this section we present a synopsis of the current data regarding the molecular processes which lead to leukostasis as observed in diabetic rodent models. Further, we attempt to synthesize these separate processes into a simplified chain of events which best represents the currently held theory of how leukostasis and inflammation lead to the vascular pathology of DR.

Many biological factors and conditions have been shown to modulate retinal leukostasis. A comprehensive list of the factors and conditions that have been experimentally shown to either induce or decrease leukocyte adhesion in the retinal circulation are
The role of leukostasis in diabetic retinopathy

shown in tables 1 and 2 respectively. The majority of leukostasis research has focused on the effects of the systemic conditions associated with diabetes as well as the effects of specific cytokines known to be increased in the diabetic milieu either systemically or locally in ocular tissues.

The hyperglycemic state induced by diabetes has been proposed to lead to DR through three main pathological biochemical processes. These are: 1) increased flux of glucose metabolites through the polyol pathway50, 2) non-enzymatic protein glycosylation and the resulting accumulation of advanced glycation end-products (AGEs)51, and 3) increased oxidative stress due to accumulation of reactive oxygen species (ROS).52 These later two process have also been implicated in increased retinal leukostasis. Systemic administration of AGEs leads directly to increased retinal leukostasis in mice53 whereas various antioxidant therapies neutralize the diabetes-induced increase in leukostasis.9

Hyperglycemia itself may also directly lead to increased leukostasis through protein kinase C-beta (PKC-β) activation54, likely via de novo synthesis of diacylglycerol.55 AGEs and oxidative stress also lead to activation of PKC-β.9,56 In human DR, a PKC-β inhibitor was shown to moderately reduce visual loss, need for laser treatment and macular edema progression in diabetic patients.57 As such, it is conceivable that PKC-β activation is a common mechanism through which AGEs, oxidative stress and hyperglycemia lead to leukostasis and DR. Inhibition of PKC-β activation has been shown to ameliorate diabetes-induced leukostasis in the rat retina.9 Activation of PKC-β leads to increased transcription of various cytokines and growth factors such as VEGF56 and the pro-inflammatory molecules TNFα and IL-1β.58,59 Each of these molecules have

<table>
<thead>
<tr>
<th>Factors increasing leukostasis</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes1,34</td>
<td>1.9 – 4.8</td>
</tr>
<tr>
<td>Increased glucose6</td>
<td>1.9</td>
</tr>
<tr>
<td>Ischemia-reperfusion35</td>
<td>90</td>
</tr>
<tr>
<td>Hyperosmolarity5</td>
<td>1.6</td>
</tr>
<tr>
<td>VEGF36,37</td>
<td>4.8-14.5</td>
</tr>
<tr>
<td>TNFa37</td>
<td>25-100</td>
</tr>
<tr>
<td>IL-1β37</td>
<td>4.5</td>
</tr>
<tr>
<td>Platelet activating factor37</td>
<td>8.7</td>
</tr>
<tr>
<td>AGEs38</td>
<td>2.7</td>
</tr>
<tr>
<td>Xanthine oxidase35</td>
<td>2.5</td>
</tr>
<tr>
<td>IFNa40</td>
<td>5.7-12.4</td>
</tr>
<tr>
<td>Insulin41</td>
<td>1.4</td>
</tr>
<tr>
<td>Hypertension42</td>
<td>2-2.7</td>
</tr>
<tr>
<td>Hypercholesterolemia53</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 1. Factors known to increase retinal leukostasis. Fold increase relative to control experiments.
The role of leukostasis in diabetic retinopathy

been shown to lead to increased vascular ICAM-1 expression as well as leukostasis in the retina.\(^{58-61}\) TNFα knock-out mice and TNFα inhibition have shown that VEGF-induced leukostasis is TNFα-dependent.\(^{58,61}\) TNFα activates the transcription factor NF-κB\(^{58}\) which in turn leads to upregulation of eNOS, FAS and ultimately ICAM-1.\(^{58,62-64}\) Leukocytes activated by the diabetic milieu adhere to the retinal vasculature and induce endothelial apoptosis through FAS-FASL interaction. It has further been proposed that this chain of events possibly leads to vision loss through two separate pathways.\(^{64}\) The first is through breakdown of the BRB via endothelial cell death in which the resulting vascular leakage then leads to macular edema. The second proposed pathway is through the accumulative effect of endothelial cell death which leads to replicative senescence and avascular capillaries. This results in areas of retinal non-perfusion and hypoxia through which VEGF expression is further increased leading to retinal neovascularization.\(^{64}\) A schematic representation of these proposed pathways is shown in Figure 1. While it is an overly simplified representation of the processes at hand, we feel it provides an adequate overview of the proposed mechanisms through which leukostasis and inflammation lead to the vascular pathology of DR.

Relevance of leukostasis in the development of DR

In the last decade compelling evidence has been accumulated suggesting DR is a disease of chronic inflammation.\(^{65}\) While this idea is not new (DR was initially termed “diabetic retinitis”)\(^{66}\), the data implicating increased retinal microvascular leukostasis as having a causal role in the development of DR has provided new support for the chronic inflammation hypothesis. While the evidence is substantial that this may be

### Table 2. Factors known to decrease retinal leukostasis. Percent decrease relative to control experiments.

<table>
<thead>
<tr>
<th>Factors decreasing leukostasis in DM</th>
<th>Percent decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiopoietin-1(^{44})</td>
<td>30 %</td>
</tr>
<tr>
<td>Aspirin(^{45})</td>
<td>100 %</td>
</tr>
<tr>
<td>COX-2 inhibitor(^{45})</td>
<td>90 %</td>
</tr>
<tr>
<td>Etanercept (anti-TNFα)(^{45})</td>
<td>100 %</td>
</tr>
<tr>
<td>Endothelin(^{24})</td>
<td>80 %</td>
</tr>
<tr>
<td>Insulin-like growth factor(^{46})</td>
<td>70 %</td>
</tr>
<tr>
<td>Valsartan(^{47})</td>
<td>33 – 60 %</td>
</tr>
<tr>
<td>Simvastatin(^{48})</td>
<td>50 %</td>
</tr>
<tr>
<td>Anti-oxidants:</td>
<td></td>
</tr>
<tr>
<td>α-lipoic acid(^{9})</td>
<td>80 %</td>
</tr>
<tr>
<td>D-α-tocopherol(^{9})</td>
<td>80 %</td>
</tr>
<tr>
<td>Corticosteroids(^{49})</td>
<td>100 %</td>
</tr>
<tr>
<td>PKC-β inhibition(^{9})</td>
<td>80 %</td>
</tr>
<tr>
<td>PPAR-γ signaling(^{34})</td>
<td>80 %</td>
</tr>
</tbody>
</table>

Factors decreasing leukostasis in DM Percent decrease

Factors known to decrease retinal leukostasis. Percent decrease relative to control experiments.
The role of leukostasis in diabetic retinopathy

In the case, a critical analysis of the current literature reveals conflicting evidence about this causal role.

TNFα-dependent VEGF-induced leukostasis was shown to be responsible for increased endothelial cell death and BRB leakage in rat retinas as early as one week after induction of diabetes.58 The TNFα-dependent nature of VEGF-induced leukostasis was demonstrated in TNFα−/− mice.61 BRB leakage was not significantly altered in these mice. The absence of TNFα and leukostasis did not prevent or reduce the amount of retinal neovascularization when these mice were used in an oxygen induced retinopathy assay.61 Moreover, SDT rats are the only rodent DR model in which retinal neovascularization occurs,67 yet they demonstrate similar levels of leukostasis as observed in other rodent models of DR.9 Taken together, these data suggest that leukostasis may not be essential for the development of proliferative DR. Leukostasis did not occur in ICAM-1−/− and CD18−/− mice with STZ-induced type 1 diabetes, up to 11 months. Retinas of these mice exhibited significantly fewer acellular capillaries and no significant decrease in the number of pericytes or endothelial cells, providing evidence that leukostasis is causal for the development of sequelae characteristic for background DR.1 In contrast, a separate study using spontaneously diabetic db/db mice, known to develop acellular capillaries at 34 weeks of age, showed no increase in leukostasis suggesting that leukostasis does not lead to acellular capillaries in this model of type 2 diabetes.12

Reasons for these contradictory results can possibly be found in the differing methodologies and diabetic models used (toxin-induced type 1 DM versus genetically inbred strain of spontaneously induced type 2 DM) or perhaps more likely in

![Figure 1. Schematic diagram of the hypothetical role of leukostasis in the development of diabetic retinopathy based on current literature. Pathways represented by dashed arrows are based on conjecture, whereas solid arrows represent pathways backed by scientific evidence.](image-url)
Table 3. Comparison of retinal lesions found in various well characterized animal models of DR, hypertension and hyperlipidemia as well as human DR. A plus (+) indicates the presence while a minus (−) indicates its absence. An empty field indicates that the lesion has not yet been reported to occur in that model. The time periods in parentheses indicate the duration of hyperglycemia in the aforementioned model.

<table>
<thead>
<tr>
<th>Model</th>
<th>Pericyte loss</th>
<th>Acellular capillaries</th>
<th>Microaneurysms</th>
<th>LB thickening</th>
<th>Capillary narrowing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human diabetes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Spontaneous diabetic rhesus monkeys (15 years)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alloxan-induced diabetic dogs (5 years)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Galactose-fed dogs (5 years)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Galactose-fed rats (23 months)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose-fed diabetic cohen rats (26 weeks)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STZ-induced diabetic rats (12 months)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Diabetic BB rat (4 months)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>OLETF rats (14 months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Zucker diabetic fatty rat (5 months)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Spontaneous diabetic torii rat (8 months)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Galactose-fed mice (26 months)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>STZ-induced diabetic mice (18 months)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>db/db diabetic mice (10 weeks)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>RICO rats (18 months)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>SHR rats (7 months)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

the genetic differences between species. This is exemplified by the variation in retinal pathology observed in the various DR models, all of which fail to replicate the entire spectrum of retinal sequelae observed in human DR (see Table 3).

In addition to the differences in size and function of ocular tissues between these species, differences in vascular patterns, cellular composition, cellular metabolism and biochemistry have also been demonstrated. Furthermore, a discrepancy in NF-κβ expression and activation exists between STZ-diabetic rats and human diabetic patients. NF-κβ is exclusively expressed in retinal vascular pericytes of human diabetics.
The role of leukostasis in diabetic retinopathy

Pericyte loss
Acellular capillaries
Microaneurysms
LB thickening
Capillary narrowing
BRB leakage
Exudates
Haemorrhages
Capillary non-perfusion
Cotton wool spots
IRMA
Neo-vascularistaion

<table>
<thead>
<tr>
<th></th>
<th>BRB leakage</th>
<th>Capillary tortuosity</th>
<th>Exudates</th>
<th>Haemorrhages</th>
<th>Capillary non-perfusion</th>
<th>Cotton wool spots</th>
<th>IRMA</th>
<th>Neo-vascularistaion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

whereas in diabetic rats its activity is increased in both retinal endothelial cells and pericytes.86 This is a crucial difference as NF-κB plays an essential role in inflammatory processes including leukostasis as well as in the retinal pathology observed in experimental diabetes through induction of various molecules such as ICAM-1 and FAS in retinal vascular endothelial cells.58,64,87 This difference in NF-κB activity in human retinal endothelial cells is supported by Hughes et al. who observed no increase in vascular expression of either ICAM-131 or FAS (unpublished data) in human diabetic retinas. The ocular differences between man and rodent are further illustrated by the
various interventions which effectively decrease leukostasis and its associated sequelae in rodents but fail to be effective at preventing or slowing progression of DR in humans.

Treatment of diabetic rats with antioxidants reduces retinal leukostasis as well as formation of pericyte ghosts and acellular capillaries. While proper randomized clinical trials involving therapeutic doses of antioxidants have yet to be performed, dietary antioxidant intake in human subjects with diabetes is not associated with a decreased incidence of DR. Aspirin therapy reduces the formation of diabetic retinal sequelae in dogs and rats but has not proven to be effective in slowing the progress of DR in humans.

Inhibition of PKC-β has also led to significant decreases in diabetes induced retinal vasculopathies including leukostasis in animal models, but it has yet to show a significant effect on preventing the incidence or progression of DR in humans. On the other hand, intravitreal corticosteroid injections decrease leukostasis and vascular leakage in diabetic rats and also improve diabetic macular edema and visual acuity in humans, which is in agreement with the hypothesis that DR is a disease of chronic inflammation.

Thus far, the only therapies proven to decrease the incidence and progression of DR in humans is strict control of hypertension and blood-glucose control using insulin or sulphonylureas. Ocular administration of insulin in rats leads to increased leukostasis and vascular pathology. This, however, is likely due to the direct effects of insulin on its many receptors in the retina, whereas the beneficial effects of systemically administered insulin in humans with diabetes are derived from its ability to lower blood-glucose levels. Studies on the effect of systemic insulin therapy on leukostasis in animal models of diabetes have not yet been performed.

Interpretation of animal DR data with respect to human DR is difficult for at least two reasons. First, are the differences between the reported outcome measurements. In animal studies these usually consist of biochemical quantitations or histochemical micropathology, whereas in human DR studies the outcomes are based on vascular macropathology and vision loss, neither of which occurs in current animal models of DR. Second, is the inability to quantify leukostasis reliably in the human retina in vivo. Until this is possible, the exact role of leukostasis in human DR will remain speculative.

Conclusion. Is leukostasis a main player in DR development or an epi-phenomenon?

Leukostasis is increased in most rodent models of DR. The evidence that it plays a causal role in the development of DR is conflicting at best. As shown in Table 1, various factors lead to very similar increases in leukostasis in the rodent retina. Retinal leukostasis is also observed in rodent models of hypertension, hypercholesterolemia and hyperinsulinemia. These diseases states also lead to various forms of vasculopathy in both the rodent and human retina, but none of them result in macular edema and vascular proliferation, causal for vision loss associated with DR. Breakdown of the BRB and vascular hypoperfusion are believed to precede these two events. Leukostasis likely contributes to retinal vascular leakage, but it is not necessary for it to occur. Increased
The role of leukostasis in diabetic retinopathy

Leukostasis does not lead to decreased overall retinal blood flow either. Local decrease in blood flow induced by leukocyte capillary plugging can cause acellular capillaries and regional hypoperfusion. One would also expect this to happen in retinas of rodents with hypertension, hypercholesterolemia and hyperinsulinemia. Leukostasis can induce apoptosis of microvascular endothelial cells and thus result in acellular capillaries and hypoperfusion. However, these sequelae are also observed in diabetic rodents without leukostasis.

Alternative mechanisms which could lead to the capillary non-perfusion observed in human DR have been proposed. Endothelial cell hypertrophy as been reported in primates treated with intra-vitreal injections of exogenous VEGF. This VEGF-induced EC hypertrophy is associated with significant capillary lumen narrowing. Increased ocular VEGF expression in diabetes could lead to widespread EC hypertrophy resulting in capillary lumen narrowing and possibly non-perfusion. Additionally, the potent vascular constrictor endothelin-1 is found in elevated quantities in the plasma of diabetic patients and leads to decreased retinal blood flow making it a plausible mechanism through which capillary non-perfusion occurs in DR.

Taken together, these findings indicate that increased leukostasis is the result of aspecific endothelial cell dysfunction as opposed to a process of chronic inflammation and is, therefore, likely an epiphenomenon of the retinal diabetic milieu. It cannot be ruled out that leukostasis enhances the pathogenic effects of the diabetic milieu on the retina. More research is needed to further elucidate the relevance of leukostasis in the development of DR in the human retina. Improved animal models of DR and the ability to quantify in vivo leukocyte dynamics in the human retina will be critical to this endeavor.

Reference List


The role of leukostasis in diabetic retinopathy


32. Duguid IG, Boyd AW, Mandel TE. The expression of adhesion molecules in the human


32

The role of leukostasis in diabetic retinopathy


The role of leukostasis in diabetic retinopathy


