Commentary

Sugar Creates a Sticky Business: Round Up the Usual Suspects

James T. Rosenbaum

From the Casey Eye Institute, Oregon Health & Science University, Portland, Oregon

In Humphrey Bogart's classic movie, *Casablanca*, Claude Rains gives orders to round up the usual suspects. In the movie, the effort is intended to be futile. In many complex diseases such as diabetes mellitus, the variety of suspects is bewildering. For diabetes those accused include serum factors such as glucose, insulin, advanced glycation end products,¹ lipids, and lipoproteins; leukocytes and the molecules that control their activation and adhesiveness;² growth factors and cytokines such as vascular endothelial growth factor (VEGF), tumor necrosis factor- α (TNF- α), insulin-like growth factor, and connective tissue growth factor;³⁻⁶ intracellular mediators such as mitogen activated protein (MAP) kinases, Akt, nuclear factor κ B, polyADP ribose polymerase, phosphatidylinositol-3 kinase, protein kinase C, and aldose reductase;^{7,8} vasoactive substances such as endothelin and nitric oxide;⁹ and oxygen radicals such as superoxide and the enzymes that control their synthesis. While the medical community's desire to control these potentially nefarious participants in the pathogenesis of diabetes is sincere, the effort to find a ringleader has been elusive. In this issue of the *The American Journal of Pathology*, Jousseaume et al¹⁰ in the laboratory of Anthony Adamis suggest that angiopoietin-1 may have the capability to corral many of these evildoers simultaneously.

The magnitude of diabetes as a health care nemesis is staggering. According to information posted on the website maintained by the National Institute of Diabetes and Digestive Diseases, ten percentage of money spent on health care in the United States is directed toward diabetes. About 6% of the population of the United States suffers from diabetes and millions of others have a recently defined metabolic syndrome with similar cardiovascular risks.¹¹ The complications of diabetes include nephropathy, retinopathy, neuropathy, and atherosclerotic disease. The vascular endothelial cell is a critical target affected by each of these complications.¹² Directly or indirectly hyperglycemia leads to endothelial cell dysfunction. One component of this is an up-regulation of

adhesion molecules with a concomitant increase in leukocyte sticking and white cell-mediated damage.²

The microvasculature of the retina is commonly affected by longstanding diabetes. Examination of the optic fundus may show cotton wool spots indicative of retinal ischemia, microaneurysms, exudates, intraretinal hemorrhages and eventually retinal neovascularization and intravitreal hemorrhage. Ischemia, macular edema, cataract, glaucoma, fibroproliferation, retinal detachment and hemorrhage may each participate in the reduced visual acuity that makes diabetes the leading cause of acquired visual loss among the working age population.¹³ What initiates this relentlessly progressive disease is often uncertain. At some point local ischemia stimulates the production of growth factors such as VEGF, which contributes further to the neovascularization and enhanced vascular permeability.

Just as it is difficult to know the major factor that initiates diabetic microvascular complications in the retina, it is difficult to identify which cell type, if any, is primarily responsible for disease. The endothelial cell is obviously a leading candidate, but pericytes, smooth muscle cells, and other cell types within the retinal milieu could each play a major or even primary role. Leukocytes are strongly implicated in the pathogenesis, since hyperglycemia leads to expression of adhesion molecules on the endothelium and the consequent leukocyte sticking occludes vessels resulting in hypoxemia. Endothelial cells represent a diverse group as demonstrated by a variety of techniques such as phage display.^{14,15} A potential limitation of many studies that seek to elucidate the pathogenesis of diabetic retinopathy is the use of endothelial cells cultured from a convenient source such as the umbilical vein rather than from the critical organ, the retina. Additionally when retinal vessels are studied, practicality often dictates that the source will be bovine or rodent rather than human.

Supported by NIH/NEI grant EY06484, Research to Prevent Blindness, and the Rosenfeld Family Trust.

Accepted for publication February 28, 2002.

Address reprint requests to James T. Rosenbaum, Oregon Health & Science University, Casey Eye Institute, 3375 S.W. Terwilliger Blvd., Portland, OR 97201. E-mail: rosenbaj@ohsu.edu.

In the report of Joussen et al,¹⁰ two animal models of streptozotocin-induced hyperglycemia are studied: recent hyperglycemia induced in rats or more chronic hyperglycemia of 16 weeks duration induced in mice. Angiopoietin-1 (Ang-1) is delivered intravitreally or systemically, in the latter case by means of an adenoviral expression system. The report focuses on the retinal vascular system. Angiopoietin-1 successfully reduces both leukocyte sticking and increased vascular permeability in the retinal vessels. While doing so, it reduces the expression of one of the most critical endothelial adhesion molecules, intercellular adhesion molecule-1 (ICAM-1). Angiopoietin-1 reduces the expression of VEGF, endothelial nitric oxide synthase, nitric oxide and the intracellular enzymes, Akt and MAP kinase. Angiopoietin-1 appears to be the ideal "prefect of police" to correct the behavior of a host of miscreants.

So what is angiopoietin-1 and why did this group choose it for study? At least four angiopoietins have been described. These proteins all share fibrinogen homology domains and coiled coils.¹⁶ Angiopoietin-1, -2, and -4 each bind the TIE-2 receptor (tyrosine kinase with immunoglobulin and EGF domains). TIE-2 is expressed on all endothelial cells. Angiopoietin-1 and -2 have antagonistic effects.¹⁷ Ang-1 induces endothelial cell apoptosis, whereas Ang-2 is a stimulus for neovascularization. Ang-1 knockout and Ang-2 transgenic mice each have major vascular defects.^{17,18} Both Ang-1 and Ang-2 are present within the retina.¹⁹ Hypoxia induces bovine retinal microvascular cells to up-regulate the expression of Ang-2 and VEGF without affecting the expression of Ang-1.²⁰ In cultured umbilical vein endothelial cells, Ang-1 can inhibit VEGF induced expression of adhesion molecules.²¹ One of the angiopoietins, Ang-3, does not influence endothelial growth, but has been identified by homology cloning.²² A recently described liver-derived protein with sequence homology to the angiopoietins, angiopoietin-like protein 3, appears to have a major role in lipid metabolism.²³

Progress has clearly been made in the treatment of diabetic retinopathy. Two NIH-funded multi-center trials, the Diabetic Retinopathy Study and the ETDRS (the early treatment of diabetic retinopathy) have demonstrated that the application of laser burns to critical portions of the affected retina benefits selected patients with diabetic retinopathy.²⁴ Vitrectomy can benefit patients whose vitreous hemorrhage is not resolving spontaneously. Improved glucose control also reduces the pace of progression of diabetic retinopathy.²⁵ Blood pressure control may also have a beneficial effect. Much of the current interest in the pathogenesis of diabetic retinopathy centers on VEGF,²⁶ which is markedly increased in the vitreous humor of patients with diabetic retinopathy.²⁷ Adamis' group has shown that a novel VEGF inhibitor is useful in an animal model of diabetic retinopathy.²⁸ VEGF is also implicated in a variety of other proliferative eye diseases, including age-related macular degeneration,²⁹ retinopathy of prematurity,^{30,31} and proliferative vitreoretinopathy.³²

The eye offers the potential for some novel pharmacological delivery systems that represent progress in strat-

egies to treat ocular diseases by local medication administration. The treatment of cytomegaloviral retinitis with an intraocular implant that could deliver the slow release of antiviral therapy for months helped to encourage the development of other slow release polymer applications. The vitreous humor behind the lens acts much like a depot to encourage a prolonged drug half-life. Local corticosteroid implants are being tested for conditions such as uveitis and macular edema; this therapy has potential application as a treatment for macular edema or other complications associated with diabetic retinopathy.³³ Photosensitizing agents are being used effectively to treat neovascularization associated with macular degeneration; their role in the treatment of diabetic neovascularization is still unclear.³⁴ Finally, the eye may be an ideal organ for gene therapy. As a quasi-closed space, novel genes could be delivered with minimal systemic risk. This strategy has been used with dramatic results in dogs with retinal degeneration.³⁵

With the impressive results reported by the Adamis group¹⁰, it is logical to ask how quickly angiopoietin 1 will join the clinical armamentarium to treat diabetic retinopathy or perhaps other forms of diabetes related microvascular disease. In this respect the limitations of the Adamis study must be considered. Obviously mice and rats are not human patients; there are some formidable examples of rodent microvascular markers differing from the parallel vascular bed in people. For example, TEM7 is a vascular marker for adenomas in the large intestine in humans but not in mice³⁶ All models, of course, have limitations as to how closely they parallel the clinical situation. Second, the study presented by Joussen et al¹⁰ examines a multitude of factors and presents data that are largely consistent and therefore convincing. But as comprehensive as the study is, it omits many factors that also contribute. For example, what is the effect of Ang-1 on other adhesion molecules such selectins, integrins, other ICAMs, and vascular cell adhesion molecule-1 (VCAM-1)? How does it affect the chemotactic factors that attract the leukocytes or the chemokines and cytokines that activate them? Third, a safe delivery system must be found with the optimal dose for efficacy and minimization of toxicity. In this regard, the eye may be the antithesis of the heart or an ischemic lower extremity. Neovascularization in the retina leads to hemorrhage, growth factor release, and vision loss. Neovascularization in coronary vessels could be life sustaining. However, Ang-1 might behave differently in different vascular beds. In contrast to its ability to inhibit VEGF-induced effects in the retina, Ang-1 promotes revascularization in a rabbit model of limb ischemia.³⁷ And finally, even if the delivery system were successful in generating solely local drug levels, toxicity could still occur? Will angiopoietin be safe such that normal retinal function is not impaired? What effect would it have in altering the response to an injury such as a retinal detachment? And what reciprocal events will occur in response to its delivery? For example, will the sustained delivery of Ang-1 produce some compensatory effect in the production of VEGF or Ang-2 that will result in toxicity?

Only a few years ago most rheumatologists would have regarded the treatment of rheumatoid arthritis (RA) as a problem somewhat similar to the challenge that now confronts ophthalmologists in controlling diabetic retinopathy. In designing treatment of RA, a biologist must consider a plethora of cytokines, growth factors, metalloproteinases, and intracellular mediators that have been implicated in the disease. The redundancy of the cytokine network is such that very few clinician-scientists had the perspicacity to predict that the inhibition of a single cytokine, TNF- α , could profoundly alter the course of the disease. By virtue of the clinical efficacy of drugs such as etanercept (a solubilized TNF receptor)³⁸ or infliximab (a humanized monoclonal antibody that neutralizes TNF- α),³⁹ the inhibition of TNF has been recognized as a master switch, perhaps *the* master switch, in the therapy of rheumatoid arthritis. The multitude of beneficial effects from Ang-1 makes it an excellent candidate to play a role in the treatment of diabetic microvascular disease comparable to the role of TNF inhibition in the treatment of RA. Only time and rigorous clinical testing will determine whether this prediction holds true. Ang-1, however, may well have the credentials to round up all of the usual suspects in what is a very sticky, complex disease.

References

- Treins C, Giorgetti-Peraldi S, Mordacca J, Van Obberghen E: Regulation of vascular endothelial growth factor suppression by advanced glycation and products. *J Biol Chem* 2001, 276:43836–43841
- Barouch FC, Miyamoto K, Allport JR, Fujita K, Bursell SE, Aiello LP, Lusinskas FW, Adamis AP: Integrin-mediated neutrophil adhesion and retinal leukostasis in diabetes. *Invest Ophthalmol Vis Sci* 2000, 41:1153–1158
- Miyamoto K, Khosrof S, Bursell SE, Moromizato Y, Aiello LP, Ogura Y, Adamis AP: Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). *Am J Pathol* 2000, 156:1733–1739
- Funatsu H, Yamashita H, Noma H, Mimura T, Yamashita T, Hori S: Increased levels of vascular endothelial growth factor and interleukin-6 in the aqueous humor of diabetics with macular edema. *Am J Ophthalmol* 2002, 133:70–77
- Giannini S, Cresci B, Pala L, Ciucci A, Franchini A, Manuelli C, Fujita-Yamaguchi Y, Cappugi P, Zeneferati R, Rotella CM: IGFBPs modulate IGF-I and high glucose-controlled growth of human retinal endothelial cells. *J Endocrinol* 2001, 171:273–284
- Lip PL, Belgore F, Blann AD, Hope-Ross MW, Gibson JM, Lip GY: Plasma VEGF and soluble VEGF receptor FLT-1 in proliferative retinopathy: relationship to endothelial dysfunction and laser treatment. *Invest Ophthalmol Vis Sci* 2000, 41:2115–2119
- Nonaka A, Kiryu J, Tsujikawa A, Yamashiro K, Miyamoto K, Nishiwaki H, Honda Y, Ogura Y: PKC- β inhibitor (LY333531) attenuates leukocyte entrapment in retinal microcirculation of diabetic rats. *Invest Ophthalmol Vis Sci* 2000, 41:2702–2706
- Soriano FG, Virag L, Szabo C: Diabetic endothelial dysfunction: role of reactive oxygen and nitrogen species production and poly (ADP-ribose) polymerase activation. *J Mol Med* 2001, 79:437–448
- Oku H, Kida T, Sugiyama T, Hamada J, Sato B, Iketa T: Possible involvement of endothelin-1 and nitric oxide in the pathogenesis of proliferative diabetic retinopathy. *Retina* 2001, 21:647–651
- Joussen AM, Poulaki V, Tsujikawa A, Qin W, Quam T, Xu Q, Moromizato Y, Bursell SE, Wiegand SJ, Rudge J, Ioffe E, Yancopoulos GD, Adamis AP: Suppression of diabetic retinopathy with angiopoietin-1. *Am J Pathol* 2002, 160:1683–1693
- Ford ES, Giles WH, Dietz WH: Prevalence of the metabolic syndrome among US adults. *JAMA* 2002, 287:356–359
- Calles-Escandon J, Cipolla M: Diabetes and endothelial dysfunction: a clinical perspective. *Endocrine Rev* 2001, 22:36–52
- Fong DS, Ferris FL, Davis MD, Chew EY: Causes of severe visual loss in the early treatment diabetic retinopathy study: ETDRS report no. 24. Early treatment diabetic retinopathy study research group. *Am J Ophthalmol* 1999, 127:137–141
- Arap W, Kolonin MG, Trepel M, Lahdenranta J, Cardo-Vila M, Gior-dano RJ, Mintz PJ, Ardelt PU, Yao VJ, Vidal CI, Chen L, Flamm A, Valtanen H, Weavind LM, Hicks ME, Pollock RE, Botz GH, Bucana CD, Koivunen E, Cahill D, Troncoso P, Baggerly KA, Pentz RD, Do KA, Logothetis CJ, Pasqualini R: Steps toward mapping the human vasculature by phage display. *Nat Med* 2002, 8:121–127
- Arap W, Haedicke W, Bernasconi M, Kain R, Rajotte D, Krajewski S, Ellerby HM, Bredesen DE, Pasqualini R, Ruoslahti E: Targeting the prostate for destruction through a vascular address. *Proc Natl Acad Sci USA* 2002, 99:1527–1531
- Conklin D, Gilbertson D, Taft DW, Maurer MF, Whitmore TE, Smith DL, Walker KM, Chen LH, Wattler S, Nehls M, Lewis KB: Identification of a mammalian angiopoietin-related protein expressed specifically in liver. *Genomics* 1999, 62:477–482
- Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD: Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997, 277:55–60
- Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD: Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* 2002, 87:1153–1155
- Hackett SF, Ozaki H, Strauss RW, Wahlin K, Suri C, Maisonpierre P, Yancopoulos G, Campochiaro PA: Angiopoietin 2 expression in the retina: upregulation during physiologic and pathologic neovascularization. *J Cell Physiol* 2000, 184:275–284
- Oh H, Takagi H, Suzuma K, Otani A, Matsumura M, Honda Y: Hypoxia and vascular endothelial growth factor selectively up-regulate angiopoietin-2 in bovine microvascular endothelial cells. *J Biol Chem* 1999, 274:15732–15739
- Kim I, Moon SO, Park SK, Chae SW, Koh GY: Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1 and E-selectin expression. *Circ Res* 2001, 89:477–479
- Kim I, Kwak HJ, Ahn JE, So JN, Liu M, Koh KN, Koh GY: Molecular cloning and characterization of a novel angiopoietin family protein, angiopoietin-3. *FEBS Lett* 1999, 443:353–356
- Koishi R, Ando Y, Ono M, Shimamura M, Yasumo M, Fujiwara T, Horikoshi H, Furukawa H: Angpt13 regulates lipid metabolism in mice. *Nat Genet* 2002, 30:151–157
- Ferris FL, Davis MD, Aiello LM: Drug therapy: treatment of diabetic retinopathy. *N Engl J Med* 1999, 34:667–678
- Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med* 2000, 342:381–389
- Benjamin LE: Glucose, VEGF-A, and diabetic complications. *Am J Pathol* 2001, 158:1181–1184
- Quam T, Xu Q, Joussen AM, Clemens MW, Qin W, Miyamoto K, Hasselmann H, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP: VEGF-initiated blood-retinal barrier breakdown in early diabetes. *Invest Ophthalmol Vis Sci* 2001, 42:2408–2413
- Joussen AM, Poulaki V, Qin W, Kirchhof B, Mitsiades N, Wiegand SJ, Rudge J, Yancopoulos GD, Adamis AP: Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. *Am J Pathol* 2002, 160:501–509
- Honda M, Sakamoto T, Ishibashi T, Inomata H, Ueno H: Experimental subretinal neovascularization is inhibited by adenovirus-mediated soluble VEGF/Flt-1 receptor gene transfection: a role of VEGF and possible treatment for SRN in age-related macular degeneration. *Gene Ther* 2000, 7:978–985
- Nunes I, Higgins RD, Aaretta L, Shamamian P, Goff SP: c-abl is required for the development of hyperoxia-induced retinopathy. *J Exp Med* 2001, 193:1383–1391
- Hellstrom A, Perruzzi C, Ju M, Engstrom E, Hard AL, Liu JL, Albertsson-Wikland K, Carlsson B, Niklasson A, Sjödel L, LeRoith D, Senger DR, Smith LE: Low IGF-I suppresses VEGF-survival signaling in reti-

- nal endothelial cells: direct correlation with clinical retinopathy of prematurity. *Proc Natl Acad Sci USA* 2001, 98:5804–5808
32. Toti P, Greco G, Motolese E, Stumpo M, Cardone C, Tosi GM: Cell composition and immunohistochemical detection of VEGF, TGF- β , and TNF- α in proliferative vitreoretinopathy. *J Submicrosc Cytol Pathol* 1999, 31:363–366
33. Jonas JB, Sofker A: Intraocular injection of crystalline cortisone as adjunctive treatment of diabetic macular edema. *Am J Ophthalmol* 2001, 132:425–427
34. Ladd BS, Solomon SD, Bressler NM, Bressler SB: Photodynamic therapy with verteporfin for choroidal neovascularization in patients with diabetic retinopathy. *Am J Pathol* 2001, 132:659–667
35. Acland GM, Aquirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J: Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* 2002, 28:92–95
36. Carson-Walter EB, Watkins DN, Nanda A, Vogelstein B, Kinzler RW, St Croix B: Cell surface tumor endothelial markers are conserved in mice and humans. *Cancer Res* 2001, 61:6649–6655
37. Shyu KG, Manor O, Magner M, Yancopoulos GD, Isner JM: Director intramuscular injection of plasmid DNA encoding angiopoietin-1 but not angiopoietin-2 augments revascularization in the rabbit ischemic hindlimb. *Circulation* 1998, 98:2081–2087
38. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ: A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999, 340:253–259
39. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P: Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999, 354:1932–1939