The Role of Growth Factors in the Development of Diabetic Retinopathy

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Proliferation of retinal blood vessels is one of the most striking features of advanced diabetic retinopathy. This feature has led to the conclusion that the normal balance of growth factors, which usually serves to keep angiogenesis in check, is disturbed in diabetic retinopathy. A considerable amount of work has been performed in the field of angiogenesis within the last decade. Much of this is applicable to diabetic eye disease, but due to the lack of an animal model, few studies have been performed directly on models of diabetic retinopathy. This review examines the literature as it relates to diabetic retinopathy.

PROLIFERATIVE DIABETIC retinopathy is one of the few examples of human pathological conditions in which abnormal growth of new vessels is one of the primary features. This observation, more than any other, has led to speculation that growth factors are involved in the pathogenesis of diabetic retinopathy in general, although at what stage in the disease process growth factors can be invoked as an influence is still far from clear. In general, angiogenesis is thought to be held in check, but every tissue must have the capability to respond with the development of new vessels as part of the process of wound healing and repair.[1] By some means, this process is disrupted in diabetes, to devastating effect in the retina. A vasoproliferative factor was proposed as long ago as 1948 by Michaelson.[2] One of the earliest candidates for this factor was growth hormone (GH), based on clinical observations,[3] but latterly it has been thought that the retina itself produces the factors responsible for new vessel growth.[4] The last decade has seen a huge amount of research on the topic of angiogenesis. Much of this is relevant to the case of diabetic retinopathy, but due to the lack of a suitable animal model, it has been difficult to extrapolate to the situation in the diabetic retina. Certain studies have examined the situation in specimens from cases of advanced retinopathy removed operatively, but the validity of this approach must be questioned. In the present review, the literature on the topic of growth factors in the pathogenesis of diabetic retinopathy is examined.

PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

The earliest changes of diabetic retinopathy consist of loss of pericytes, capillary dilatation, capillary leakage, and an increase in basement membrane thickness. In the context of growth factors, several of these changes could have far-reaching consequences. Pericytes are thought to have a contractile activity, maintaining normal capillary tone. Loss of pericytes and increased capillary blood flow could alter the configuration of capillary endothelial cells, altering their susceptibility to normally present growth factors.[1] Doubtless, the pericytes also produce growth factors themselves, and their loss could contribute to the early changes of retinopathy. This has been confirmed in a study in which pericytes and smooth muscle cells grown in cultures allowing contact with endothelial cells inhibited growth and multiplication. In the absence of contact but with free access of diffusable factors, this inhibition is lost.[5] This suggests that pericytes produce an inhibitor of endothelial cell growth, and further investigation has suggested that this inhibition is mediated through secretion of activated transforming growth factor-β (TGF-β).[6] Clearly, therefore, pericyte loss in early diabetic retinopathy is not without consequence, although whether the above mechanisms are operative in vivo remains to be seen.

Notionally, thickening of the extracellular matrix and alteration in its chemical composition are also of extreme importance. In culture, the nature of the extracellular matrix has important consequences for cell growth and morphology,[7-10] and it is highly likely that this is also true in vivo. In diabetes, the documented changes in chemical composition of the basement membrane include increased nonenzymatic glycosylation of collagen, decreased levels of heparan sulfate proteoglycans, and variable reports on changes in fibronectin and laminin.[11] Such changes might alter cell-cell contact and perhaps allow cellular invasion and breakdown of the basement membrane, one of the earliest features of angiogenesis. Additionally, since a number of growth factors are bound and activated by heparin, the alterations in heparan proteoglycans may be of relevance, although direct evidence is awaited. The importance of composition of the basement membrane is being increasingly realized, and research on the role of the matrix protein, thrombospondin, in inhibiting angiogenesis has suggested another area worthy of investigation in the context of diabetes.[12]

On a biochemical basis, a number of current hypotheses have implications for the activity of growth factors. One such proposes that as a consequence of hyperglycemia there is de novo synthesis of diacylglycerol,[13] which in turn is an activator of protein kinase C (PKC). Precise details have yet to be clarified, but this pathway has been demonstrated to be operative in retinal endothelial cells in culture[14,15] and in rat glomeruli.[16] The implications of this for the initiation of angiogenesis remain highly speculative. However, PKC has been shown to induce cellular invasion of the matrix on which the cells are grown in culture[17] and to increase cell growth in general.[18,19] PKC has been proposed as one of the postreceptor mediators of insulin and insulin-like growth factor-I (IGF-I) action,[20,21] and

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probably of many other growth factors and hormones alike. If this mechanism could be demonstrated to be operative in vivo, it would suggest that the activity of many growth factors could be altered as a result of diabetes, with consequences for the maintenance of the normal balance of growth factors in vascular tissues.

The hypothesis that advanced-glycosylation end products are formed on proteins with a long half-life also has hypothetical implications for the actions of growth factors.[22] Apart from changes in the basement membrane as a consequence of glycation of type IV collagen that have been discussed earlier, certain growth factors could also be released as a result of increased traffic of mononuclear cells. These cells normally bind to endothelial receptors,[23] but it is proposed that glycosylation of these receptors increases binding, leading to an increase in the influx of mononuclear cells.[24] Histological studies have demonstrated that such an influx does occur in diabetic retinopathy.[25] The consequences of this, apart from vascular occlusion, would include release of growth factors that might mediate invasion of the extracellular matrix, and stimulation of endothelial cell growth. Indeed, the activities of certain growth factors, such as TGF-β and tumor necrosis factor-α, to stimulate angiogenesis are thought to rely on the influx of inflammatory cells.[26]

The later stages of retinopathy, culminating in proliferation of new vessels, are generally thought to be mediated by generation of growth factors produced by the ischemic retina. Although definitive proof of this is lacking, it certainly seems to be almost self-evident. One of the principal goals of research into the pathogenesis of diabetic retinopathy is to identify the growth factor responsible for vascular proliferation, and as will be discussed later, there are now candidates that can make a good case for involvement. However, as a point of interest, it is worth noting that the growth factor or factors responsible for vascular proliferation in diabetic retinopathy need not necessarily be produced by vascular cells. The energy from photocoagulation is taken up only by pigmented materials, which in the case of the retina will be the retinal pigment epithelial (RPE) cells. Photocoagulation therefore has its major effect on RPE cells and the outer retina, and yet is patently effective in the treatment of proliferative retinopathy. A number of researchers have examined the ability of RPE cells to influence endothelial cell growth with conflicting results.[27,28] However, it is clear that any contender for the vasoproliferative factor in diabetic retinopathy will need to take RPE cells into account.

SPECIFIC GROWTH FACTORS IN THE DEVELOPMENT OF DIABETIC RETINOPATHY

The most intensive research in the field has focused on the quest for the growth factor responsible for proliferative retinopathy. Clinicians have long fostered an interest in IGF-I, but this probably arises because this is an easily measured circulating growth factor. The basis of the hypothesis is that hypophysectomy or pituitary ablation was found to be an effective treatment for proliferative retinopathy before the advent of photocoagulation.[29-38] Taken with the observation that diabetes is associated with elevated GH levels[39,40] and that GH is the only major pituitary hormone not routinely replaced after ablation or hypophysectomy, a case was made for GH as the angiogenic factor in diabetes.[3] With the development of assays for IGF-I, it has become apparent that there is no difference in circulating levels in individuals with and without retinopathy,[41-43] although increased levels of IGF-I have been reported in the vitreous of patients undergoing vitrectomy for severe retinopathy.[44,45] On a scientific basis, IGF-I is not usually associated with angiogenesis, although there have been reports that in experimental models it can induce this response.[46] On theoretical grounds, it is possible to make a case for the involvement of IGF-I in the development of early retinopathy, although, paradoxically, it was in proliferative retinopathy that hypophysectomy was found to be effective.

More promising as the vasoproliferative growth factors were acidic and basic fibroblast growth factor (aFGF and bFGF). These heparin-binding growth factors are potent stimulators of angiogenesis.[47] FGF was found in preretinal membranes, vascular basement membranes, and vitreous of patients with advanced diabetic retinopathy,[48-50] although only in small amounts in new vessel fronds. However, the FGFs do not fit the bill perfectly as the major angiogenic factor in diabetic retinopathy, in that they do not have a signal peptide and are therefore released only in response to cell death or injury. One would therefore have to suppose that some form of injury consequent to glucose toxicity damages the endothelial cells, bringing about release of FGF, with perhaps altered binding by the chemically modified basement membrane leading to increased activity or availability. However, a recent report has suggested that FGF is glycosylated in bovine retinal endothelial cells grown in hyperglycemic conditions, with consequent loss of heparin binding and mitogenic activity.[51] This observation requires further investigation, but casts further doubt on the role of FGF in diabetic retinopathy.

Perhaps more promising as the angiogenic factor of diabetic retinopathy is the family of vascular endothelial cell growth factors (VEGFs), of which four have been identified.[52] VEGF is a potent and specific endothelial cell mitogen. In the context of diabetic retinopathy, it has the attraction of being upregulated by hypoxia. This fits in well with the proposed hypothesis that increasing retinal ischemia stimulates production of an angiogenic factor, leading to new vessel growth. VEGF has been reported to be present in increased amounts in the aqueous and vitreous of patients with all grades of diabetic retinopathy, particularly those with proliferative disease.[53] It is likely that there will be an increasing literature on VEGF and diabetic retinopathy in the next few years. In view of the actions of VEGF to increase capillary permeability, there is likely to be interest in these growth factors with respect to diabetic maculopathy.

Of the other known growth factors, the literature with regard to diabetic retinopathy is patchy. The inhibitory effect of TGF-β on vascular proliferation has been described earlier. There have been studies of platelet-derived growth factor (PDGF) in the context of diabetes in general,

without specific reference to diabetic retinopathy. Although reports have varied, the majority opinion would appear to be that levels of PDGF in blood and platelets of diabetic subjects do not differ from levels in nondiabetic subjects.[54-57] One important recent finding is the fact that receptors for PDGF are present in endothelial cells of rabbit retina,[58] but the case for involvement of this growth factor in the development of retinopathy has yet to be made. Other growth factors, such as platelet-derived endothelial cell growth factor, have received no attention with regard to diabetic retinopathy, and there are still a large number of avenues awaiting exploration.

CONCLUSION

Due to the patchy nature of the research in this area to date, it is difficult to construct a working model of the involvement of growth factors in the pathogenesis of diabetic retinopathy. The problem lies in making the huge leap from basic vascular biology in animal models and cell culture studies to the situation in human diabetes. Based on the information we have to date, it is possible to propose only a sketchy outline. Presumably, direct glucose toxicity leads in some fashion to early endothelial cell damage, death of retinal endothelial pericytes, and thickening of the capillary extracellular matrix. This disrupts cell-cell contact, with alteration in the balance of growth factors that maintain angiogenesis in check in the normal retina. Loss of inhibitory control of angiogenesis by TGF-β from pericytes would be an example of this process. Furthermore, biochemical changes consequent to hyperglycemia might alter secretion or activity of various growth factors. Influx of mononuclear cells due to glycosylation of endothelial cell receptors may mediate activity of certain indirect angiogenic factors such as tumor necrosis factor-α, and platelet aggregation may release platelet-associated factors. Basement membrane abnormalities and cell injury may release angiogenic factors such as FGF. With increasing retinal ischemia, increasing amounts of VEGF may be expressed, leading finally to capillary leakage and new vessel formation. Many details in this model are highly speculative, and all are open to further scrutiny. It is hoped that the next decade will provide some further answers to the vague perceptions we have at present.

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