CHAPTER 10

GENERAL CONSIDERATIONS AND FUTURE PROSPECTS

It is still not clear whether ARM is an exaggeration of the normal aging process, or a fundamentally different disease entity. Photoreceptor cell loss appears to occur in both instances.[26,90] Our finding of apoptosis in the aging retina can be explained by Wallace's theory of aging and disease, in which cells die at reaching a threshold by both genetic and environmental damages.[24] The time of onset could be influenced by genetic factors, or by environmental factors. Apoptosis can be initiated by multiple stimuli. In ARM, apoptosis is most likely the common pathway of cell death, resulting for instance from cellular damage, growth factor withdrawal or other stimuli. If one cell is damaged beyond repair, the apoptotic program is activated in order to prevent damage to the surrounding tissue. In this view, the finding of apoptotic cell death in specimens with ARM may state more about the quality and quantity of damage than about the general way of cell death in ARM. In my opinion, without restoring RPE function, the effect of anti-apoptotic modalities in the treatment of ARM will be limited. Anti-apoptotic interventions could leave severely damaged, probably non-functional retinal cells.[336]

The RPE seems to play a central role in the pathogenesis of ARM. Protective and shielding qualities are allocated to the RPE.[64,133] With aging, the RPE may lose some of these qualities, allowing neovascularization. Fas-ligand, expressed on the RPE, is proposed to be one of those protective factors, inducing apoptosis of proliferating vascular endothelial cells.[64] We showed in the study on Fas-ligand that the RPE does not have a decreased Fas-ligand expression with age. Furthermore, we demonstrated that Fas-ligand expression on RPE cells in sub-RPE CNV is similar to the expression in subretinal CNV, in which the vessels grow through the RPE into the subretinal space . These results make Fas-ligand less likely to be a major suppressive factor of the RPE, in case a CNV already has developed.

Malfunctioning RPE, age-related thickening of Bruch's membrane, BLD and other factors may eventually lead to neovascular AMD, possibly by relative hypoxia of the retina. In 1948, Michaelson proposed the presence of a diffusible biochemical "factor X" in the eye that was capable of inducing angiogenesis in diabetic retinopathy. The last two decades, numerous growth factors have been acknowledged in the

pathogenesis of neovascular retinal disease. VEGF has appeared to play a central role in the process of ocular angiogenesis. However, the precise mechanism of VEGF in the complex interaction of the different angiogenic growth factors in AMD has not been elucidated so far. Other angiogenic growth factors seem to play additional roles. In this thesis, we detected the presence of the Insulin-like Growth Factor (IGF) family in neovascular AMD. It is established that IGF-I has angiogenic properties in ocular vascular endothelial cells.[173] Therefore it is possible that IGF plays a role in the pathogenesis of neovascular AMD. While VEGF may control angiogenesis by acute oxygen regulation, IGF-I might do so on the basis of availability of nutrients.[177] IGF-I is recruited in normal wound repair,[276,337] that may partly explain the presence of IGF-I in CNV, because formation of the disciform lesion is regarded as normal wound repair.[1,74,119,120,275,276] On the other hand, IGF-I may function as a trophic factor for the normal vascular system.[338] With increasing age and consequently decreasing IGF-I levels,[339] the vascular endothelial cells may experience a decreased protective effect of IGF-I,[338] resulting in vascular insufficiency and thus further hypoxia in the outer retina, increasing the chance of angiogenesis.

For AMD the exact role of the IGF family and its possible therapeutic properties are still unclear. In order to study the role of IGF-I the individual IGF family members should be quantified in CNV and surrounding retinal tissue and related to values in normal tissue. Focusing on the dynamic role of the IGF family in CNV formation, it is mandatory to develop CNV in models of transgenic mice over- and underexpressing IGF-I, IGF receptor type 1 and the various IGFBPs.

Somatostatin and analogues such as octreotide seem to be candidates for inhibition of ocular angiogenesis.[177] They inhibit the secretion of growth hormone in the hypopituitary. Somatostatin seems to have further repressing effects on angiogenesis such as downregulation of VEGF in RPE cells[274] and anti-proliferating effects on vascular endothelial cells,[173,174,181] possibly mediated by somatostatin receptors.[173] These effects can be used as a tool to treat neovascular AMD. We demonstrated somatostatin receptor subtype 2A, which has high affinity for octreotide, in neovascular AMD. Therefore, local treatment could also be an option. Two further effects attributed to somatostatin can be of help in order to improve visual acuity in patients with neovascular AMD. Firstly, the drainage effect on macular edema of somatostatin,[340] and secondly the excitation of neuronal cells,[300] which could be directly associated with an increase of visual acuity. A randomized controlled phase II trial using octreotide in patients with neovascular AMD is currently under study.[183]

In view of the current assumption that angiogenic growth factors act in concert, anti-angiogenic treatment of patients with neovascular AMD addressing only one growth factor may be overruled by other growth factors. Therefore, it is likely that in the future a combination of pharmaceuticals mediating different growth factors will be applied as a therapy.

The view of the author on the pathogenesis of ARM is reflected in a schematic illustration in Figure 10.

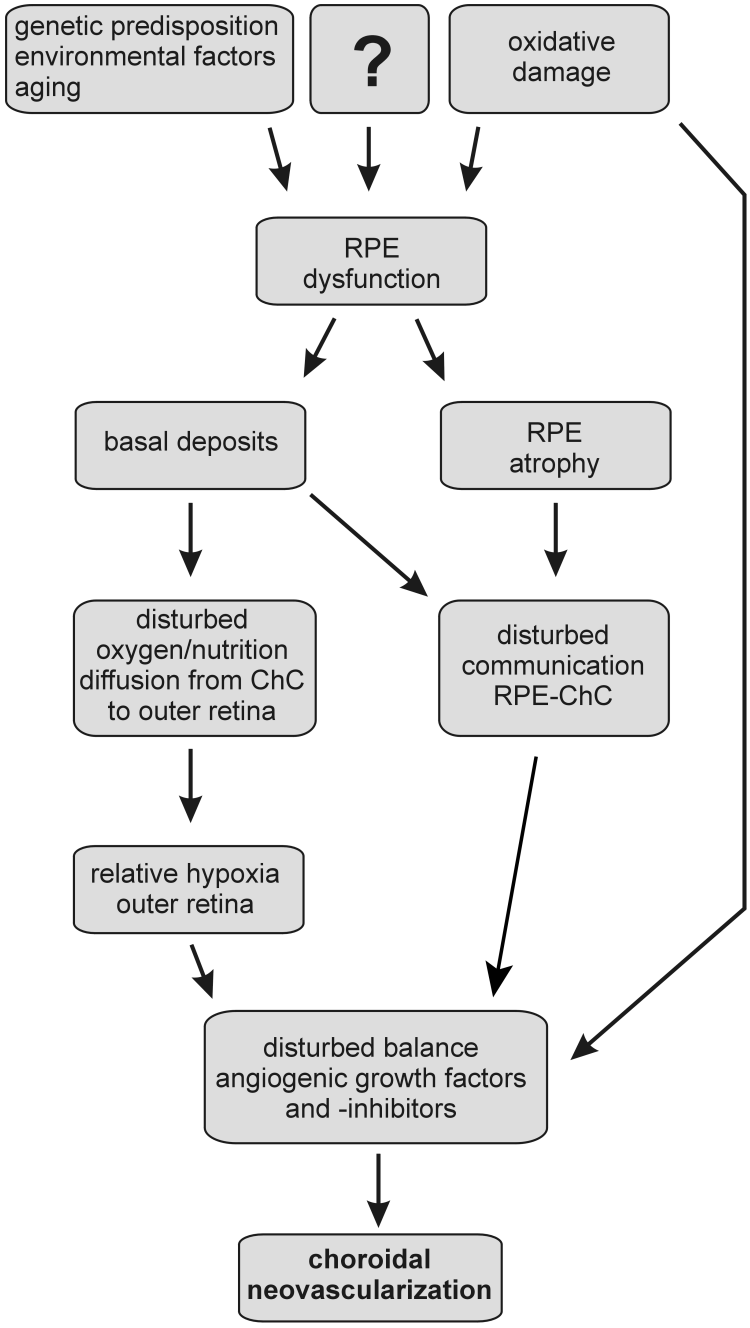

***Figure 10. Simplified schematic illustration of pathogenesis of ARM**, leading to choroidal neovasculari-zation.*

A major drawback of the research on neovascular AMD on paraffin-embedded human eyes is the scarcity of material. Patients with ARM rarely donate their eyes at autopsy for further research. Furthermore, eyes donated for corneal transplantation purposes rarely have signs of advanced ARM in the posterior segment. Most studies on human material are therefore performed on surgically removed (small) subretinal membranes.

In addition, a study performed on paraffin embedded material of neovascular AMD is static research. However, the CNVs we studied reflect different stages of the development of the disease, with the assumption that a mixed or subretinal membrane (which in AMD often is a part of a mixed membrane) is a progression of a sub-RPE membrane into the subretinal space.[120]

Because of the scarcity of human material, in vitro models like co-cultures[341] and collagen gels[192,341] can be used. However, in order to test the hypothetical models of the pathogenesis of ARM, a dynamic approach with animal models is mandatory. Currently there is no efficient suitable animal model of ARM available. Only some animals have the macular anatomy that is comparable to the human macula, such as the non-human primates, as we described in Chapter 4. Rhesus monkeys (or *Macaca mulatta*) show changes similar to early ARM such as drusen, but end stage AMD rarely occurs in these animals.[231] It may be hypothesized that the RPE characteristics are different in monkeys, or that the richly pigmented choroid contains more antioxidants than human choroid. In black people, having a more pigmented choroid, features of early ARM are common, but advanced AMD is infrequent, compared to Caucasians.[342]

Mice have the advantage of fast aging, but the drawback of mouse models of CNV is the absence of a macula. In order to produce CNV in animals, retinal damage is induced by for instance laser treatment. However, this may not represent 'normal' conditions in which ARM develops in humans. In another animal model of CNV, VEGF expression in the RPE is upregulated.[161-164] Still in these models RPE probably functions normally, thus not all aspects of ARM are addressed. Additionally, in experimental CNV, budding capillaries are rapidly enveloped by proliferating RPE, followed by an involution of new vessels.[133] This could explain the self-limiting disease that often occurs in animal experiments of CNV. The ideal animal model for neovascular AMD should be a fast aging animal with a macula, and a dysfunctional RPE, since this seems to be critical in the pathogenesis of AMD.