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Role of growth factors and the wound healing response in age-related macular degeneration

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Abstract Growth factors (GF) are important in several stages of the pathogenesis of age-related macular disease (AMD). In choroidal neovascularization (CNV) in exudative AMD, the GF involved are similar to those involved in wound healing of the skin. Like granulation tissue of skin, CNV is characterized by clotting, inflammation, angiogenesis and fibrosis, and like in skin wounds, members of the VEGF, angiopoietin, PDGF and TGF- β families of GF are expressed. However, several of these GF may also serve physiological functions in the normal eye, where the retinal pigment epithelium (RPE) employs them to provide trophic support to the neuroretina and choriocapillaris, in addition to maintaining an anti-angiogenic state. De-arrangement of these physiological

functions may underlie the initiation of CNV in AMD. Basolateral secretion of VEGF-A by the RPE maintains the choriocapillaris, and is enhanced by hypoxia. Age-related changes in Bruch's membrane lead to impairment of this trophic function and choriocapillaris atrophy, as well as to decreased diffusion of oxygen towards the neuroretina. The resulting outer retina hypoxia may be an important driving force of CNV formation, by stimulating VEGF overexpression by the RPE, in addition to the effects of increased oxidative stress and low-grade inflammation. RPE senescence and hypoxia may also decrease expression of angiogenesis inhibitors such as PEDF, further shifting the balance to a pro-angiogenic state in the aging eye.

Introduction

Age-related macular disease (AMD) [8] often causes loss of vision in the elderly. A better understanding of the pathogenesis of this complex disease is needed in order to develop better preventive and interventional treatment strategies. Such strategies may be directed at the array of growth factors that have been shown to be involved in the development of AMD.

Growth factors, neurotrophic factors, chemokines and cytokines (GF) are cell-secreted mediators of autocrine and paracrine functions, involved in cell maintenance, survival, growth and death, as well as in angiogenesis, vascular permeability, inflammation and other processes [37, 69]. Most GF were originally thought to have single

functions in one of these processes, but often, their functional potential has broadened. An example is vascular endothelial growth factor-A (VEGF-A), which was originally described as a vascular permeability factor, then identified as being important in angiogenesis, whilst recent work has highlighted the roles of VEGF as a mediator of leukocyte adhesion in vascular cells and as a neurotrophic factor in the central nervous system [11, 70].

Inhibition of VEGF in exudative AMD has recently been reported to benefit patients [63], confirming experimental evidence that GF cause choroidal neovascularization (CNV). However, angiogenesis inhibition alone will not be sufficient to restore the function of a macula affected by AMD, as in addition to CNV, the clinical and pathological spectrum of AMD shows a variety of

changes. These range from drusen deposition and pigment epithelium detachments to scar formation [8]. Increased oxidative stress has been identified as a central mechanism in AMD pathogenesis [7].

Many GF have been implied in AMD, mainly based on experimental data obtained from studies in cultured retinal pigment epithelium (RPE) and from histopathological studies in surgically removed human CNV or in laser-induced CNV in transgenic rodent models [16, 21, 50]. These studies have identified the RPE not only as an important source of growth factors involved in tissue maintenance, homeostasis, inflammation and CNV formation, but also as the central cell type acting in and regulating these processes [7, 9, 21].

These considerations are especially relevant for the scientific field that tries to explore transplantation of the RPE as a therapeutic approach in AMD. The purpose of this review is to provide an overview of our present knowledge of the role of GF and the RPE in AMD.

Growth factors and wound healing: the angiogenic balance

Histological studies show that CNV represents wound healing tissue quite similar to the granulation tissue (GT) of skin wounds [19, 20]. The role of GF in wound healing has been most extensively studied in such skin wounds and genuine granulation tissue. The enormous number of GF shown to be involved in this process (Table 1) and their highly complex interactions have been excellently reviewed elsewhere [37, 69]. The wound healing response in skin is characterized by inflammation, angiogenesis and fibrosis and serves to restore the integrity of the tissue. Distinct phases and a number of key processes are recognized, including (blood) clotting, formation of a provisional fibrin matrix, infiltration by neutrophils and macrophages, angiogenesis, extracellular matrix formation, scarring and reepithelialization [69]. By histology, distinct zones can be recognized in human GT, consisting of an outer fibrin layer, a superficial layer with abundant vascular sprouting, an intermediate layer with vascular maturation and exten-

sive matrix formation, and a deep layer characterized by fibrosis [56]. In addition to monocytes, circulating cells such as fibrocytes and endothelial progenitor cells may also contribute to the formation of granulation tissue [1, 58, 69]. Initially, these invading cells and platelets are a main source of growth factors, but later in GT development, GF production by macrophages, (myo)fibroblasts, endothelial cells, pericytes and keratinocytes becomes more important [69]. The exact temporal and spatial involvement of all these GF in GT formation has only been partly elucidated (Table 1) [69]. In GT angiogenesis, members of the VEGF family, released by macrophages and platelets are considered important in the initial migration and proliferation stages of vascular and lymphatic growth. PDGF-BB produced by endothelial cells helps to recruit pericytes, and angiopoietin-1 produced by pericytes acts on endothelial cells through its receptor Tie-2 to form stable mature vessels (Fig. 1) [37]. It has been suggested that in resting tissues many potent angiogenesis factors such as FGFs and VEGFs are present, but that these are counteracted by constitutive expression of angiogenesis inhibitors such as thrombospondins. This angiogenic balance would initially swing towards angiogenesis during GT formation and would be restored after termination of the wound healing response [15, 28, 69].

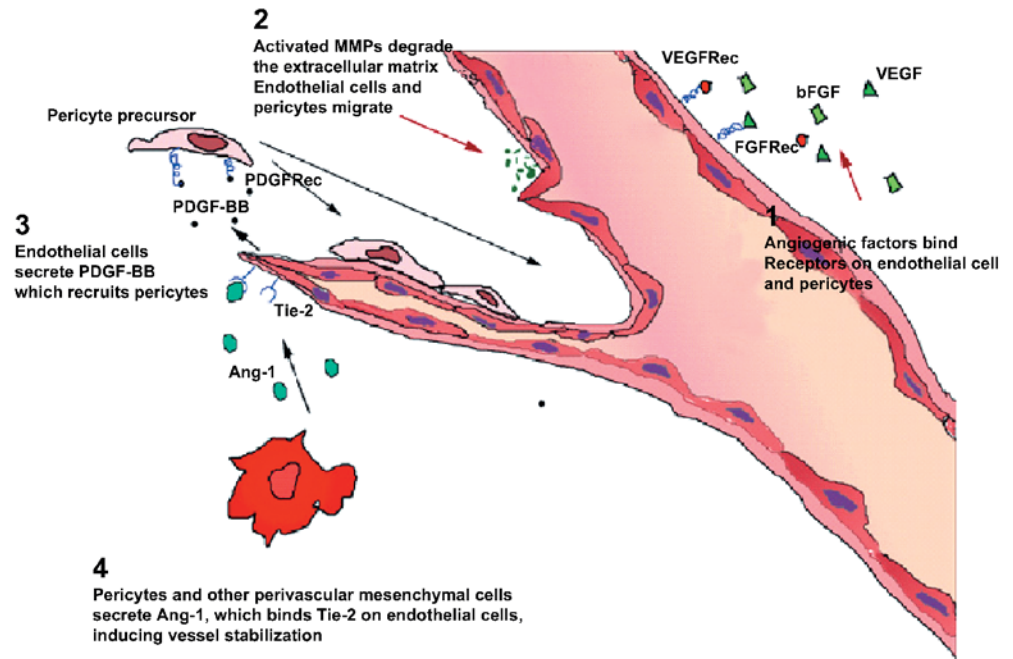
Experimental models for AMD research

How does CNV in AMD compare to skin wound healing? AMD research typically lacks adequate models allowing investigation of the different stages of the disease. Cultures of human RPE cells have been used extensively. In interpreting results of these studies, it is important to realize that RPE cells have the potential to transdifferentiate into a wound healing phenotype, expressing functions completely different from the highly differentiated non-dividing RPE cells within their monolayer in the normal eye [25, 74]. Tissue culture conditions are likely to provide an environment inducing this wound healing phenotype. By specific measures, RPE cells in vitro can be made to differentiate into a phenotype resembling RPE in vivo, displaying polarity, a columnar

Table 1 Growth factors and cytokines and their functions in skin wound healing and choroidal neovascularization [69]. *Bold type* indicates GF demonstrated in CNV tissue; (-) negative regulation

Process	Growth factors involved
Neutrophil infiltration	TGF-β , MCP-1 , MIP2/GRO- α , IL-8, IL-6, IL-10(-)*
Macrophage infiltration	TGF-β , MCP-1 , MIP-1 α , IL-10(-)
Angiogenesis	VEGF-A , PLGF , FGF2 , angiopoietins , HGF , Cyr61, MCP-1 , IL-8, GRO- α , GM-CSF, IP-10(-)
Fibroplasia	PDGF , TGF-β , CTGF , GM-CSF, IGFs
Matrix deposition	FGF2 , IGF-1 , NGF, TGF-β , activin, MCP-1 , CTGF , Cyr61
Scarring	IGF-1 , TGF-β , activin, CTGF , IL-6, IL-10(-)
Reepithelialization	FGF2 , FGF7, FGF10, EGF, TGF- α , HB-EGF, NDF, IGFs , NGF, activin, MCP-1 , IL-6, GM-CSF, leptin, TGF-β (-), BMP-6(-), IP-10(-)

Fig. 1 Schematic representation of the step-wise development of angiogenesis and of the main growth factors involved



morphology, the presence of gap or even tight junctions, and the presence of microvilli at their apical surface [9, 46, 74]. Unfortunately, many publications fail to describe the differentiation state of the studied RPE cells, rendering the findings of these studies often useless. RPE cells may be cultured on filter systems, allowing evaluation of polarized GF secretion, or in co-culture systems with choriocapillaris (CC) endothelial cells or neuroretinal explants [9]. Exposing the cells to substances relevant to the pathogenesis of AMD, such as rod outer segments (ROS) or advanced glycation end products (AGE), provides ways to investigate mechanisms involved in AMD, such as lipofuscin formation and oxidative stress [7].

Recent work has identified transgenic mouse models with retinal changes such as basal linear deposits, reminiscent of human non-exudative age-related maculopathy, and these models may teach us more about the contribution to AMD of mechanisms like oxidative stress [4, 51]. Transgenic mice which lack or overexpress a specific GF gene, in particular in a specific tissue or under a conditional control system, are also useful in investigating which GF are involved in CNV formation. Such models can be combined with the laser CNV model, which has also been applied in rats and monkeys, where local destruction of the RPE and Bruch's membrane by laser causes a CNV-like response [50, 65]. This response can be semi-quantified and be studied by immunohistochemistry, by in situ hybridization, or by PCR mRNA analysis of the CNV tissue isolated with laser capture dissection [50]. Finally, several studies have investigated GF expression and cellular content of surgically removed human CNV.

The RPE in embryonic development

What can we learn in AMD research from the normal eye and its development? In ocular development, the choroid forms from two embryonic tissues, the mesoderm and the cranial neural crest cells. All cells in the choroid derive from neural crest cells except for the endothelial cells. The pattern of development suggests that interactions between the primordial RPE and the periorcular mesenchyme are important, and in line with this notion it was shown that in the absence of an RPE layer, the choroid does not develop [73]. High expression of VEGF in the embryonic RPE, and of its receptors VEGFR-1 and -2 in the periorcular mesenchyme, in particular in a layer of cells adjacent to the RPE, has been demonstrated [71, 73]. In human eyes as well as in rats and mice, VEGF mRNA levels in RPE cells in vivo have their peak expression during the embryonic stage of development of the choroid, but expression persists into adulthood [71, 72, 73]. This suggests that VEGF is one of the factors involved in choroidal development driven by the embryonic RPE, a paracrine relation that may persist throughout life to maintain the CC. In vitro, RPE cells are particularly sensitive for hypoxia-induced VEGF overexpression [2, 60]. Choroidal development via VEGF expression in embryonic RPE may therefore be driven by hypoxia, resulting from retinal thickening and the high oxygen demand of the developing photoreceptors.

Growth factors and the RPE in the normal eye

Clinical and experimental observations suggest that also in the normal adult eye, the RPE cell layer is crucial for maintenance and survival of both the neuroretina and the choroid (reviewed in [70]). Destruction of the RPE by laser invariably leads to atrophy of the CC and photoreceptors. This is also supported by the finding that experimental destruction of RPE cells causes atrophy of the CC, which is preceded by loss of fenestrations of the CC endothelium [69, 70]. Experimental data show that GF are involved in this physiological function of the RPE and its interactions with the retinal cells and CC. One function of the RPE may be to lend trophic support to the retina by production of neurotrophic GF such as nerve growth factor (NGF), brain-derived growth factor (BDNF) [29] and pigment epithelium-derived factor (PEDF) [29, 30]. BDNF may also promote differentiation of the RPE itself in an autocrine manner [22]. PEDF, a 50-kDa secreted glycoprotein, is an important neurotrophic factor for retinal cells [30] produced by the resting RPE. In vitro, PEDF is mainly produced by highly differentiated RPE cells [45]. In addition to its neurotrophic properties, PEDF is a potent inhibitor of angiogenesis [13]. PEDF may contribute to maintaining the angiogenic balance in ocular tissues, as it is downregulated in human and experimental conditions with ocular neovascularization, such as proliferative retinopathy and exudative AMD [26, 45, 54]. Thrombospondin-1 (TSP-1) is another potent angiogenesis inhibitor, produced by RPE cells in vitro and demonstrated by immunohistochemistry in resting RPE in vivo [43], that the RPE employs to maintain an anti-angiogenic state in the resting choroid. A similar role has been suggested for members of the TGF- β family [42].

In addition to these angiogenesis inhibitors, the resting RPE has been convincingly shown to produce the pro-angiogenic VEGF-A, both in vivo and in vitro. In vitro, VEGF production is considerably higher in differentiated than in undifferentiated RPE cells, a finding that supports a physiological role of VEGF-A [9, 45]. In vitro, VEGF secretion by highly differentiated RPE cells was shown to be directed preferentially towards the basal side of the cells [9], whilst in vivo in the adult human choroid, the VEGF receptors 1, 2 and 3 are expressed by the CC endothelium on its side facing the RPE cells [9]. As VEGF can act as a survival factor for endothelial cells [3], and as it induces a fenestrated permeable endothelial phenotype [14], these findings indicate that VEGF-A is involved in the physiological maintenance function of the resting RPE on the choroid [9]. Expression of VEGFR-3, a ligand for VEGF-C and -D but not for VEGF-A, in the human CC in vivo [9, 70], together with the recently demonstrated constitutive expression of placenta growth factor (PlGF) in mouse choroid [50], suggests involvement of other members of the VEGF

family in this function as well. In addition to this role of GF, it was shown in an in vitro model that the extracellular matrix deposited by cultured RPE cells, and the presence of basic fibroblast growth factor (bFGF) within this matrix, may also contribute to CC survival by inhibition of endothelial apoptosis [38]. The presence in the choroid of potent angiogenesis inhibitors such as PEDF and TSP-1 may counteract the angiogenic effects of VEGFs and bFGF on the CC.

In differentiated RPE cells in vitro, VEGF-A upregulates PEDF expression via VEGFR-1 in an autocrine manner [46]. Although this observation suggests complex interactions between these pro- and anti-angiogenic factors to maintain tissue homeostasis in the normal human retina, expression of VEGF receptors by human RPE in vivo has not yet been convincingly demonstrated [9, 73]. Finally, RPE cells in vitro also secrete angiotensin (Ang)-1 and -2 [23]. These GF have been implied in the later stages of angiogenesis, acting in stabilizing and remodeling of newly formed vessels (Fig. 1) [64]. Ang-1 is produced by perivascular cells and the receptor for Ang-1 and -2, Tie2, is constitutively expressed by endothelial cells, suggesting a role of angiotensins in normal vascular function such as regulation of permeability (reviewed in [64]). In cultured RPE cells, Ang-1 and -2 were found to be expressed and VEGF-A was shown to upregulate Ang-1 secretion by these cells in an autocrine manner [23]. As these cultured RPE cells were of unspecified differentiation it remains unknown whether Ang-1/Tie2 interactions are involved in CC homeostasis in the resting eye.

Taking the published findings together, in the normal eye complex GF networks are employed by the RPE to play a central role in supporting the surrounding tissues and maintaining an anti-angiogenic state.

Pathology of CNV and cell types involved

CNV in conditions other than AMD is usually of the classic type as seen by fluorescein angiography (FA), anatomically corresponding to fibrovascular tissue growing between the retina and RPE (Figs. 2, 3 and 5). In AMD, several types of CNV are recognized [18], and their pathogenesis may be different. By FA, most cases have occult CNV, a situation where the neovascular activity is primarily located between the RPE and Bruch's membrane [35, 36]. In the course of time, the overlying RPE cells migrate into these lesions or degenerate, and the membrane may proceed in growing directly under the neuroretina. Other cases initially only have classical membranes or demonstrate both types of CNV in an early stage of the disease. In AMD, the arrest of growth of CNV, by the RPE or by other mechanisms, seems to fail. This leads to continuing growth of the lesion, far beyond meaningful wound healing, and the eventual scar

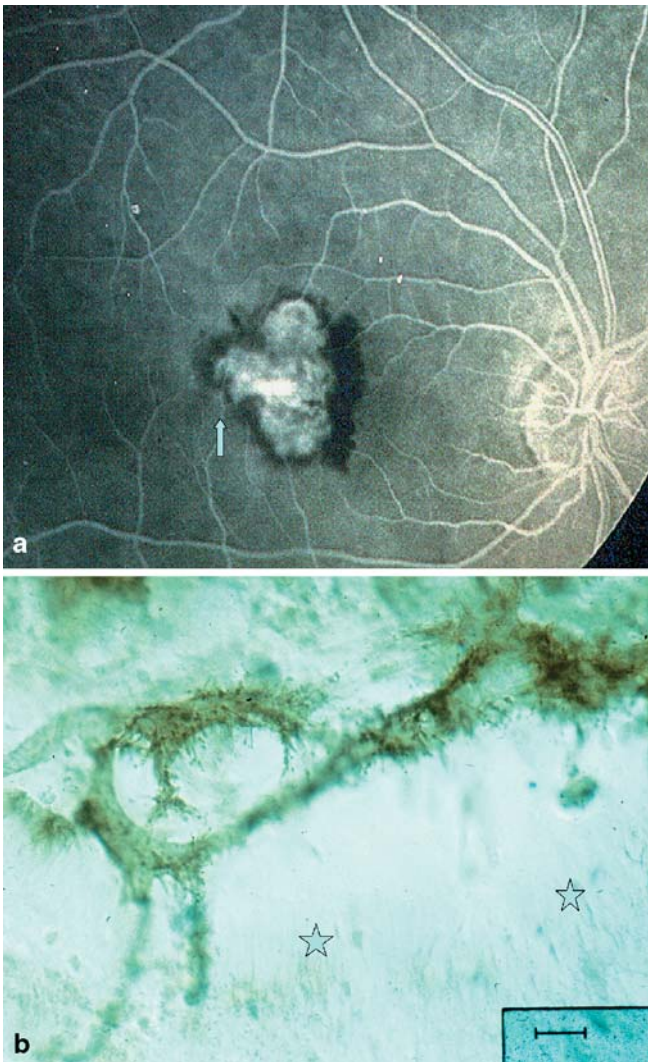


Fig. 2A,B Clinico-pathological correlation of idiopathic CNV. Note in **A** the hypofluorescent rim surrounding the hyperfluorescence of the leaking membrane which probably represents a front of fibrin. The *arrow* points at a complex of neovascular sprouts which can also be recognized in the histological specimen (**B**). Note in **B** the numerous small endothelial extensions typical of actively sprouting capillaries invading the fibrin matrix (*asterisks*)

may take up a large portion of the posterior pole. In addition, there are specific age-related subtypes of CNV such as polypoidal choroidal vasculopathy and retinal angiomatous proliferations (RAP). Whether different GF are involved in these various types of CNV is unknown. It is likely, however, that once CNV is developing, an array of GF expression and function occurs that is shared by wound healing elsewhere [17,20]. The histological key components of the wound healing and GT response, i.e., inflammation, angiogenesis (Fig. 2) and fibrosis, and the corresponding cell types, i.e., macrophages, newly formed vessels and (myo)fibroblasts,

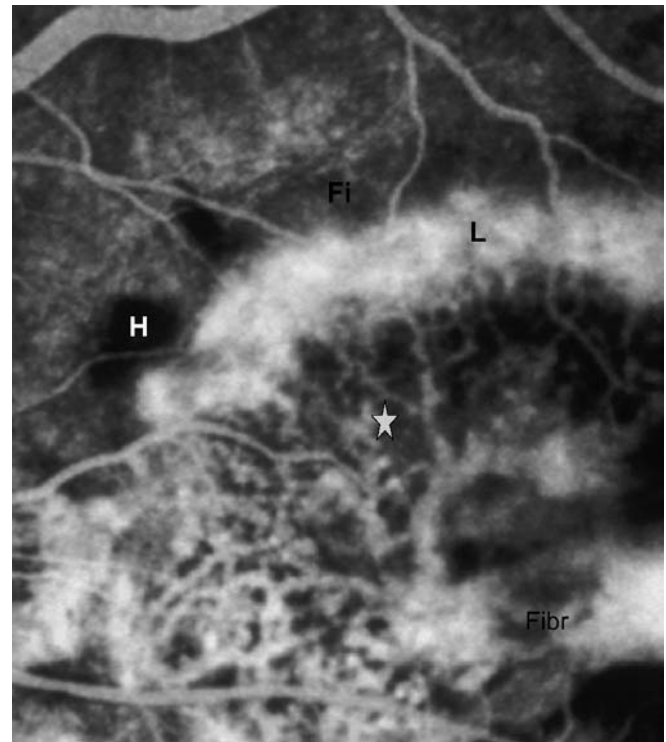


Fig. 3 Stages in development of CNV, reminiscent of the layered structure of granulation tissue in skin wound healing. Note from top to bottom the hypofluorescent peripheral rim of fibrin (*Fi*), the actively leaking front of growing vessels (*L*), the central part of the lesion characterized by less leaky, maturing vessels (*asterisk*), and an area with fibrosis (*Fibr*). *H* indicates hemorrhage

have all been demonstrated in human and rodent CNV in histopathological studies [17,21]. In experimental rodent CNV, systemic macrophage depletion reduced the size of the lesions, indicating the important role of these cells in CNV formation [55]. The clinical heterogeneity and variable degree of eventual subretinal fibrosis in human CNV are likely to reflect a variable contribution of the histological key components of GT to the evolution of CNV. By histology, some authors distinguish inflammatory from non-inflammatory CNV [21], differences that probably reflect the stage of evolution of the wound healing response. Some CNV membranes are associated with hemorrhage, and this will not only introduce GF derived from platelets and leukocytes, but will also expand the provisional fibrin matrix, which may in itself stimulate further vascular growth. In the absence of hemorrhage, human CNV in its classic form is also associated with a front of fibrin [36], visible on fluorescein angiography (FA) as a hypofluorescent rim around the hyperfluorescent leaky membrane (Figs. 2 and 3). Behind this fibrin layer, FA often shows a front of actively growing, highly leaky vascular sprouts (Figs. 2 and 3), developing into less leaky vessels in the center of the lesion (Fig. 3). These characteristics are reminis-

cent of the layered structure and growth patterns of GT in skin wounds.

Growth factors in human and experimental CNV

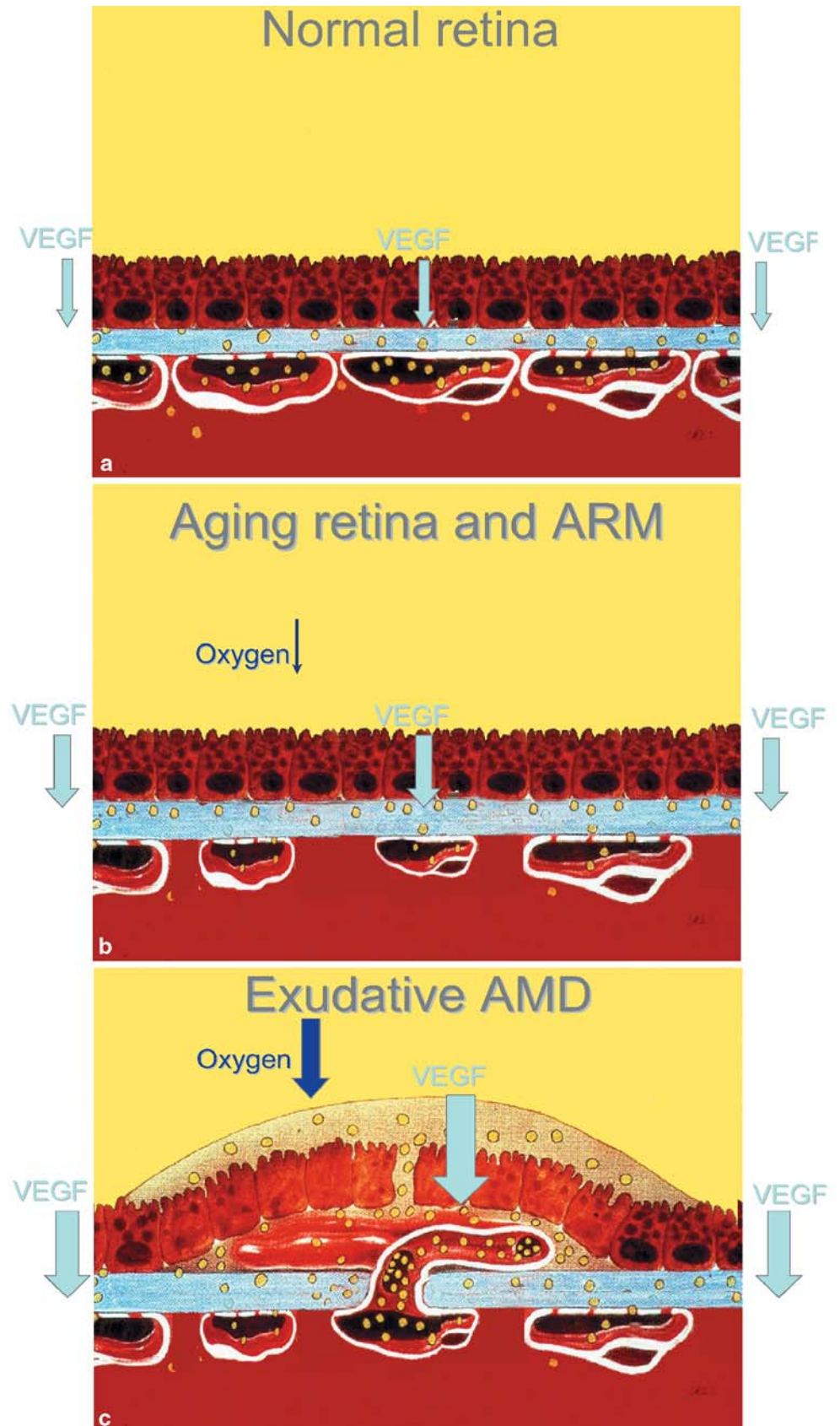
It is not surprising that within established CNV membranes, many of the GF known to be involved in skin GT have been demonstrated (Table 1) [5, 21, 53]. How these GF contribute to the different stages of evolution of CNV is largely unknown. A specific characteristic differentiating CNV from wound healing elsewhere is the presence of RPE cells in various degrees of transdifferentiation [17, 19]. In patients with a retinal detachment, such transdifferentiated RPE cells are known to cause proliferative vitreoretinopathy (PVR), another ocular wound healing condition. In PVR, involvement of a number of GF has been implied, including hepatocyte growth factor/scatter factor (HGF), PDGF-B, TNF, TGF- β and connective tissue growth factor (CTGF) [25]. The involvement and relative contribution of these factors to CNV formation needs to be determined. RPE cells within CNV membranes were shown to express, among other things, monocyte chemotactic protein (MCP) [21], a molecule involved in macrophage recruitment, and members of the VEGF and angiopoietin families of GF [23, 34, 39]. The VEGF family consists of several GF acting on three types of receptors. VEGFs have important and varying roles in vascular permeability, angiogenesis and lymphangiogenesis and have neurotrophic and pro-inflammatory functions (reviewed in [70]). In migrating RPE cells and macrophages in human and experimental CNV membranes, VEGF-A, -B, -C and -D, PlGF and VEGFR-1 (Flt1) and -2 (Flk1) have been demonstrated at the protein and mRNA levels [16, 34, 39, 48, 50, 68]. In murine CNV, the smallest isoform of VEGF-A, VEGF-A₁₂₀, was the main type of VEGF-A expressed [50]. These observations have led to (pre)clinical studies of VEGF-A inhibition in patients with CNV [63]. In some of these patients and in preclinical models partial regression of CNV lesions was observed, and this has confirmed that VEGF-A has an important role in CNV growth [33, 63]. Experimental studies in rodents have thrown some light on the relative contribution of VEGFs to this process. Overexpression of VEGF-A in the RPE or in photoreceptors by viral transfection leads to CNV formation, but only if some form of local trauma is induced [57, 62]. In the absence of trauma, VEGF-A overexpression in the RPE causes increased choroidal vascular permeability and leukocyte adhesion [57]. Studies in VEGF-A knockout mice are not possible, as these animals are not viable. However, PlGF knockout mice develop normally, but the loss of PlGF inhibits pathologically increased permeability and angiogenesis in cancer and retinal ischemia in these mice [40]. It has been suggested that PlGF, through interaction with its receptor

VEGFR-1, specifically enables survival of newly formed vessels [59]. This mechanism may operate in CNV development, as experimental CNV lesions induced by laser in PlGF knockout mice were smaller than in wild-type controls [50]. In addition to VEGFs, the two main members of the angiopoietin family of GF, Ang-1 and Ang-2, were also demonstrated at the protein level in human CNV tissue in migrating RPE cells, suggesting involvement of these GF in vascular maturation, stabilization and remodeling in CNV [23, 47]. Recently, expression of CTGF in human CNV was reported [24]. CTGF is a pro-fibrotic factor also capable of inducing angiogenesis [61]. Which of these functions is important in CNV development needs to be determined.

Role of the RPE in the pathogenesis of CNV

Ascertaining why CNV starts growing in AMD is one of the main challenges in this field of research. CNV can occur in a large number of ocular conditions. In most of these, there is some kind of defect in Bruch's membrane in the presence of a viable retina, accompanied by a variable degree of inflammation [49]. The defect in Bruch's membrane may be clinically visible, as in pigmentary changes and atrophy in AMD, chorioretinal scars, traumatic choroidal ruptures, angioid streaks or myopic lacquer cracks. Increased oxidative stress is an important mechanism in the pathogenesis of AMD [7], reviewed elsewhere in the January 2004 issue of this journal. Increased oxidative stress is likely to lead to overexpression of GF by the RPE and a pro-inflammatory state, causing invasion of macrophages resulting in local destruction of Bruch's membrane [7, 49]. Indeed, macrophages have been demonstrated along the outer side of Bruch's membrane in eyes with drusen [21], possibly attracted by RPE-derived MCP [21]. However, hypoxia may play a role in the development of CNV as well [12]. Based on our findings in the normal eye we have suggested a possible scenario for the pathogenesis of CNV in AMD (Fig. 4) [9]. As previously discussed, RPE cells in the normal eye predominantly secrete VEGF-A at their basolateral side, towards the CC, maintaining the CC and inducing its fenestrated permeable phenotype. In vitro, hypoxia markedly increases VEGF-A secretion by RPE cells at their basolateral side [9]. Thus, the maintenance function of the RPE on the CC may be characterized by a hypoxia-driven feedback mechanism. It is tempting to speculate that in aging, but in AMD in particular, this paracrine relation is disturbed by a thickened Bruch's membrane, which is less permeable to water-soluble compounds such as VEGFs due to the accumulation of lipids (reviewed in [8]). Because of these changes in Bruch's membrane, VEGF-A may not be able to reach the CC in order to support it. This would result in CC atrophy, which has indeed been observed in aging eyes [41, 52], and hypoxia of the outer

Fig. 4A–C Schematic representation of the possible role of VEGF and hypoxia in the pathogenesis of CNV in AMD. In the normal retina, VEGF secreted by the RPE to its basal side maintains the choriocapillaris (A). In aging, thickening of Bruch's membrane inhibits diffusion of VEGF and oxygen, leading to choriocapillaris atrophy and outer retina hypoxia (B). Hypoxia causes VEGF up-regulation and accumulation, leading eventually to neovascularization through localized defects in Bruch's membrane (C)



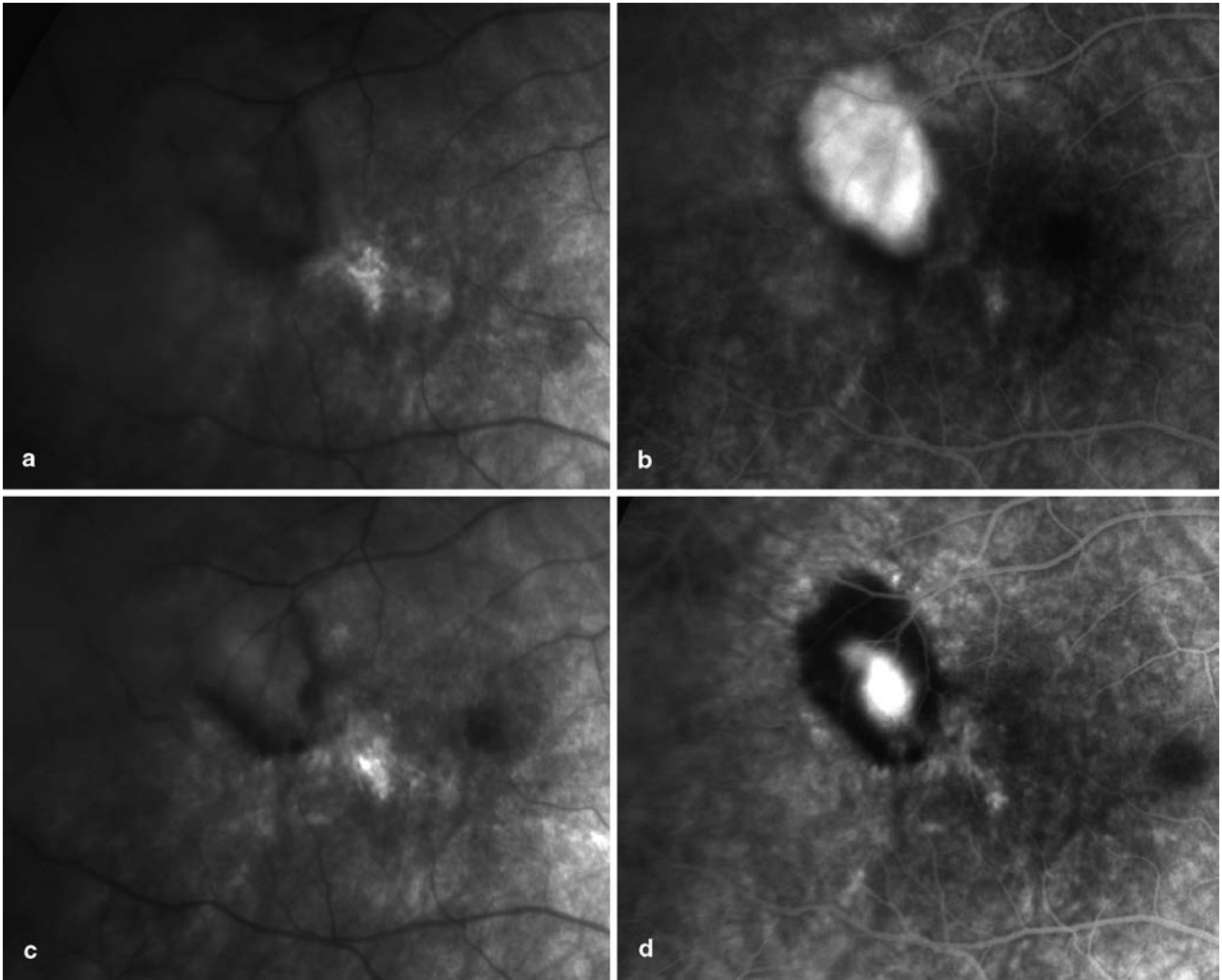


Fig. 5A–D Role of the retinal pigment epithelium in the involution phase of CNV. **A,B** Note the actively growing CNV membrane in a myopic patient. **C,D** Four months later, spontaneous involution has occurred, associated with a ring of pigment around the lesion

retina. Outer retina hypoxia may be further enhanced by decreased diffusion of oxygen from the choroid to the RPE and retina, caused by the age-related changes in Bruch's membrane [8, 10, 27]. This is supported by recent studies indicating decreased diffusion of ethanol, a molecule only slightly larger than oxygen, through Bruch's membrane in eyes with AMD [6].

Thus, the aging changes in Bruch's membrane may cause hypoxia in the outer retina from CC atrophy and decreased oxygen diffusion [8]. The RPE will respond with increased basal secretion of VEGF [9], which will accumulate due to the barrier properties of the thickened, lipid-laden Bruch's membrane [27]. In the presence of

localized defects in Bruch's membrane, caused by atrophy or activity of macrophages [49], CNV may be initiated by these accumulations of high amounts of VEGF (Fig. 4). Histopathological and experimental observations support this concept: immunostaining of VEGF-A is increased in and around the RPE in the macula of patients with age-related maculopathy, a condition with a high risk of developing CNV [32]. Experimental overexpression of VEGF-A by the RPE, in the presence of a surgical trauma, induces CNV [12, 62]. Based on this scheme of events, CNV may have some functional significance in AMD, as it provides the hypoxic tissues with a new blood supply. Selective therapeutic destruction of CNV, as is done with photodynamic therapy (PDT), may therefore increase the stimulus for neovascularization, a pattern indeed suggested by the clinical course of some PDT-treated patients.

As described above, VEGF-A is secreted by the resting RPE. In addition, VEGF-A, PlGF and all three

VEGF receptors were demonstrated in the normal choroid and neuroretina, suggesting not only (possibly neurotrophic) physiological functions of VEGFs in the eye, but also the necessary presence of mechanisms counteracting the pro-angiogenic and permeability effects of these GF. Thus, other biochemical factors, such as oxidative stress or a decrease in angiogenesis inhibitors, and anatomical factors, such as damage to Bruch's membrane, must occur to allow the development of CNV. In this respect a role of PEDF in CNV pathogenesis has been suggested [44, 45]. In the vitreous of patients with neovascular AMD, PEDF is decreased [26]. In the rat model of laser-induced CNV, where VEGF-A protein is strongly upregulated, it was shown that expression of PEDF is decreased in cells within CNV tissue [54]. In contrast, in a later stage, PEDF was strongly expressed in RPE cells sealing the CNV, suggesting that PEDF secreted by the RPE cells is involved in regression of CNV.

Thus, the RPE probably initiates the CNV response, but may also be involved in its arrest. Encapsulation of the fibrovascular membrane by a monolayer of RPE cells, comparable to reepithelialization in skin wounds, is observed in histopathological studies [66] and can often be seen clinically in young patients with CNV (Fig. 5). In CNV tissue, FasL, an apoptotic molecule, is expressed in the RPE cells and may control angiogenesis beneath the retina. The growing vascular endothelial cells in CNV express the apoptosis-signaling receptor Fas and are sensitive to Fas-mediated cell death [31]. Therefore, in addition to a role of PEDF, FasL expression from the RPE may attenuate CNV by killing the Fas-sensitive vascular endothelial cells in the vicinity [31].

Growth factors and RPE transplantation

The goal of RPE transplantation is to restore sight in patients with AMD. It is intended to lead to a new monolayer of highly differentiated, fully functional RPE cells, resting on a (provisional) Bruch's membrane, and a CC.

Experimental approaches in RPE transplantation have ranged from applying cell suspensions to using autologous sheets of RPE with the underlying Bruch's membrane [67]. Considering the potential of RPE cells to change rapidly into a wound healing cell, the latter approach seems desirable. RPE cells in a suspension, detached from their basement membrane, are likely to produce unwanted GF at the site of transplantation, a wound bed by itself, and decrease their anti-angiogenic and trophic functions. In contrast, autologous RPE sheets with the Bruch's membrane still intact may allow the cells to maintain their differentiation and physiological functions and preserve the associated anti-angiogenic state.

Conclusions

Growth factors are crucial in AMD. This role of GF may start as early as in embryonic development, when the choroid develops in the vicinity of the fetal RPE [73]. The mechanisms by which the RPE induces choroid formation, and the factors that attract the massive number of capillaries developing into the CC, may have a related role in adult life [9]. In aging, these mechanisms may fail or become deranged and contribute to the development of disease. An additional role of the RPE lies in control of local inflammation, and inflammation is likely to play a role in the sequence of events leading to CNV [19, 20]. The main physiological function of CNV may be to rapidly restore the integrity of the ocular wall, rather than preserve sight, and this may be the reason for the significant collateral damage to visual function inflicted by CNV. Once established, CNV formation runs an independent course, like GT formation in skin wounds. In AMD, this course is expanded by failure of inhibitory mechanisms. Several of the main GF involved in CNV formation also have important functions in the normal healthy eye, and this may have implications for therapeutic approaches aimed at CNV.

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