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Involvement of immature endothelial cells in vascular alterations in Alzheimer's disease

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Abstract

The aim of the present study was to investigate ultrastructural features of cerebral capillaries and the pattern of new vessel formation in a patient with Alzheimer's disease (AD). Recent neuropathological studies have demonstrated that patients with AD have cerebrovascular pathology. Using electron microscopy, we showed that alterations of the capillaries are a common finding both in vascular disease and in AD, suggesting that vascular factors may also play a role in the pathogenesis of AD. We also found regionally increased capillary density, and in many sections immature endothelial cells lying on the preexisting endothelium were present in the lumen of capillaries. These cells might thus contribute to the pathological pattern of capillaries. The cytoplasm of immature endothelial cells in the patient with AD was characterized by accumulation of amyloid fibrils. We suggest that immature endothelial cells may be an important source of circulation-derived amyloid in the brain.

Key words: *Alzheimer's disease, amyloid fibrils, endothelial cell, angiogenesis, ultrastructure.*

Introduction

The development of neurodegenerative disorders such as Alzheimer's disease (AD) is generally associated with a wide range of histological and pathophysiological alterations. Although the diverse triggers of the neurodegenerative processes and their interactions are still the topic of extensive debate, possible contribution of cerebrovascular deficiencies has been suggested in the recent years. Alzheimer's disease has emerged as one of the great mysteries in modern medicine. Although none of the prevailing theories about the genesis of AD has resolved the mystery, each has led to intriguing findings suggesting the need for further investigations. AD is multifactorial, with both genetic and environmental factors implicated in its pathogenesis [4,22].

For a number of years two major hypotheses regarding the cause of AD were proposed. According to the amyloid cascade hypothesis the neurodegenerative process is a series of events triggered by abnormal processing of the amyloid precursor protein [1,14]. The neuronal cytoskeletal degeneration hypothesis assumes that cytoskeletal changes are the triggering events [32].

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Much research on AD has focused on determining the mechanisms underlying the toxicity associated with Aβ proteins [25]. A major area of research into Aβ peptide toxicity is induction of inflammatory response following the release of pro-inflammatory cytokines that trigger an inflammatory cascade resulting in neuronal death [24]. Aβ peptide toxicity involves free radical formation. Synthesis of reactive oxygen species, which initiate cellular changes leading to apoptotic cell death, is regulated by multiple factors like calcium and inflammatory cascades [23,24].

Recent studies suggest an important role of blood vessels in the pathogenesis of AD. The microvascular aspects of neurodegenerative processes in AD were described, with special attention to cerebral blood flow, neuronal metabolic changes and the abnormalities of microvascular ultrastructure [8].

Numerous structural and functional alterations of the cerebral microvasculature in AD include increased microvascular density. More recently, a hypothesis regarding a crucial role of endothelial cells as mediators of progressive destruction of cortical neurons and pathologic angiogenesis in AD [33] caused much debate among investigators. This hypothesis points to neovascularization in the brain in AD that occurs in response to impaired cerebral perfusion and vascular injury. Morphological and biochemical evidence for this process include regionally increased capillary density, vascular loop formation, glomeruloid vascular structure formation and expression of angiogenic factors. The authors suggested that angiogenic activation of the brain endothelium in AD leads to deposition of β-amyloid plaques [33]. Recent findings implicate that perivascular neurons, astrocytes and vascular cells constitute a functional unit, acting together to maintain tight control of the biochemical composition of the brain parenchymal environment [16,38]. Multiple pathogenic cascades originating from altered cerebral blood vessels can initiate dysfunction of the neurovascular unit, including aberrant angiogenesis, cerebral amyloid angiopathy and senescence resulting in increased levels of Aβ [38].

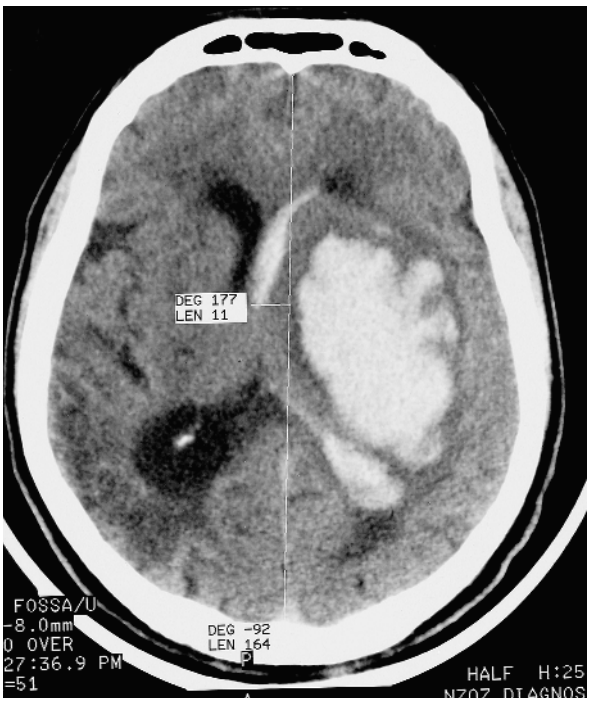

Fig. 1. CT of the head before the operation

Neovascularization during adult life has long been attributed to angiogenesis only. Studies on angiogenesis have revealed that adult bone marrow is a rich reservoir of endothelial progenitors. Mobilization and recruitment of these cells is essential for tissue revascularization in ischemic hindlimbs, ischemic myocardium, cutaneous wounds and tumor vasculature [2,21,30,31] in a process called postnatal vasculogenesis. In animal models of brain ischemia, immature endothelial cells were found to incorporate into sites of active neovascularization [2,18,27,35].

The aim of this study was to analyze the brain tissue from a patient with AD at the ultrastructural level using electron microscopy. We present evidence that vascular pathology in AD may be related to vascular progenitors.

Material

The patient, a 67-year-old woman, was urgently admitted to the Department of Neurosurgery in a very poor clinical status due to spontaneous intracerebral haematoma in the left cerebral hemisphere.

Initially the patient was admitted to the Neurological Department in a regional hospital with suspected ischemic stroke. The patient had been treated for 4 years until that moment in a neurological outpatient clinic following the diagnosis of AD. CT of the head (Fig. 1) was performed due to worsening of the neurological status of the patient,

leading to the diagnosis of intracranial haematoma and the patient was transferred to the Department of Neurosurgery.

On admission her clinical condition was critical, scoring 7 points in GCS, with localizing response to pain only. A decision to perform emergent neurosurgical procedure was made.

During the operation craniotomy was performed in the fronto-parietal area of the skull, and the haematoma was reached and removed through the cortex of the left cerebral parietal lobe.

While making an access through the cerebral cortex, a small resection of the cerebral cortex was made due to surgical technique and the removed fragment was sent for microscopic examination.

The clinical condition of the patient did not change after the surgery and she remained unconscious and intubated in a poor neurological status with hemiplegia.

When the surgical wound healed, the patient was transferred to the regional Neurological Department. She remained in a critical condition, which unfortunately did not improve and after 4 weeks the patient died in the Neurological Department.

Methods

The material was processed for ultrastructural morphological studies using transmission electron microscopy and analyzed with JEM-1200EX as described earlier [9].

Results

The ultrastructural analysis of the fronto-temporal cortex specimens of our case revealed the presence of randomly oriented 7.5 to 9 nm amyloid fibrils in neurons and axons. In addition a large fibrillar deposit extending into the adjacent neuropil was observed (Fig. 2). Amyloid fibrils consisted of hollow rods and were composed of filaments arranged as a tightly coiled helix. At higher magnification they showed bead-like structure. The neurofibrillar tangles and dense bodies were observed in some parts of dystrophic neurites (Fig. 3). The severe damage of the capillary walls has been observed in the fronto-temporal cortex of the affected patient. Microvascular changes were manifested by the pericytes and basement membrane abnormalities. Pericytes in our material showed signs of degeneration, including extensive

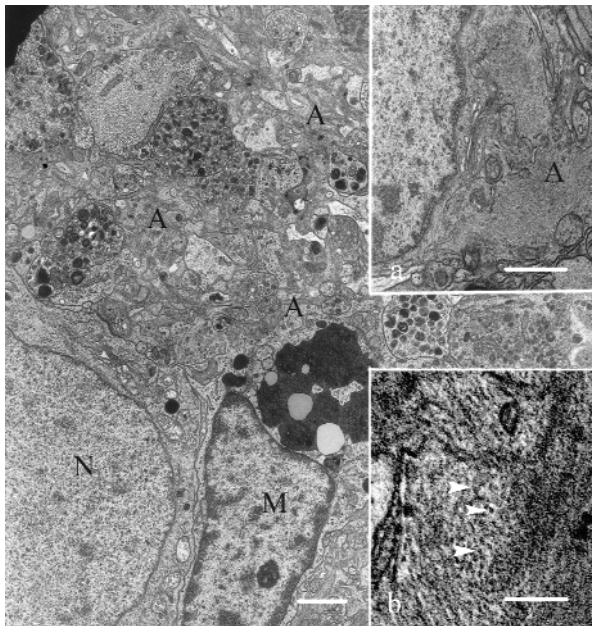

Fig. 2. The ultrastructural features of analysed brain cortex. Pericaryal part of a neuron (N) with a microglia (M) cell in the vicinity. The brain parenchyma is characterized by neuropil degeneration. Deposits of amyloid (A) are seen among neuropil elements. Bar 2 µm. Insert a. High magnification of amyloid fibrils (A) in a neuronal cell. Bar 1 µm. Insert b. High magnification of amyloid fibril in transverse sections (arrowheads). Bar 200 nm

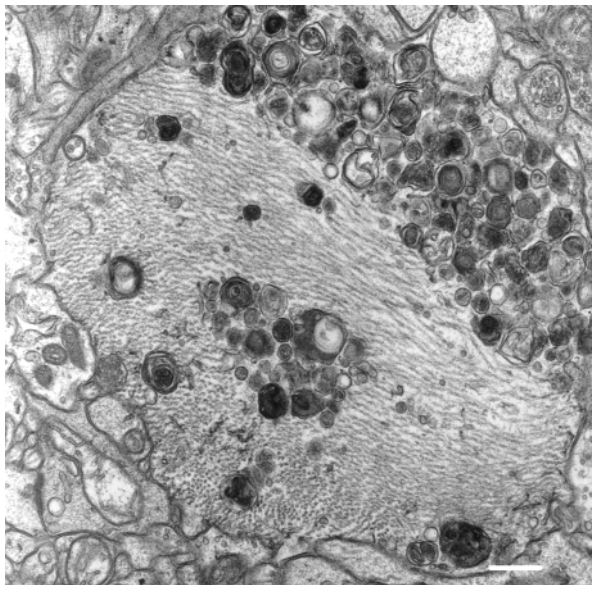

Fig. 3. Ultrastructural features of dystrophic neurites with neurofibrillary tangles and many dense bodies. Bar 500 nm

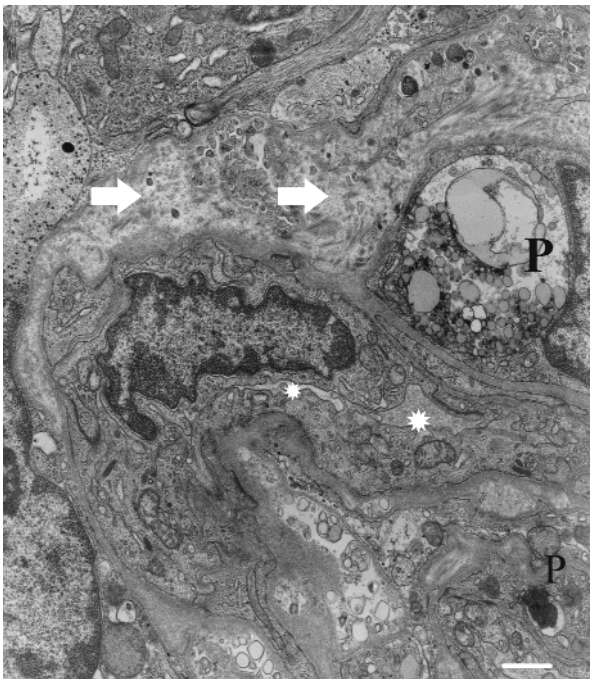

Fig. 4. The young blood vessel with high hypertrophied endothelium and narrow vessel lumen (asterisks) is surrounded by basement membrane with collagen fibrils (arrows). Pericytes (P) show signs of degeneration of cytoplasm with deposits of lipids and granular dense material. Bar 1 µm

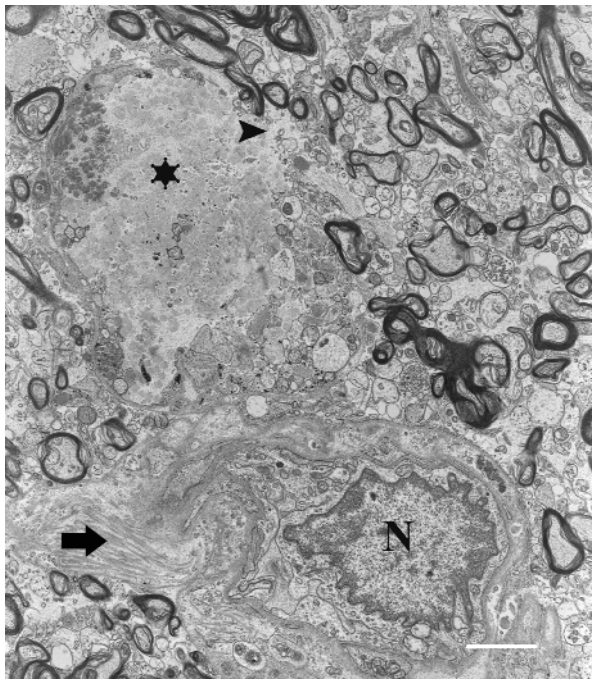

Fig. 5. The blood vessel with characteristic pathological changes including thickening and disruption of the basement membrane (arrowhead) and accumulation of collagen fibrils (arrow and asterisk). Atypical AD features of the endothelial cell nucleus (N) are seen. Bar 2 µm

deposition of lipids and granular dense material in phagolysosomes (Fig. 4). The basement membrane thickening probably due to the accumulation of basement membrane proteins and collagen deposition (Fig. 5) within basement membrane were the most frequent findings in investigated areas. The transverse diameter of the collagen fibrils was approximately 40 nm and the periodicity of fibrillar striation was 64 nm. The giant forms of collagen fibrils with a diameter about 100 nm, showing irregular profiles on transverse sections have been also observed in enlarged spaces between capillary vessels and the brain parenchyma. In some areas immature endothelial cells have been noted in small capillaries. They covered the surface of remaining endothelial cells (Fig. 6). In addition immature endothelial cells leaving the vessel lumen were observed in close proximity with the capillary wall. They were characterized by the presence of fine cytoskeletal fibrils and exhibited amyloid fibrils which decorated basement membrane-like material (Fig. 7).

Cytoskeletal fibrils (a characteristic feature of the immature endothelial cell) and amyloid fibrils are morphologically different. Amyloid fibrils are seen as structureless material or poorly defined short rods or fuzzy fibrils (Fig. 7), whereas cytoskeletal fibrils form a regular dispersed network of intermediate filaments. Our observations show that the immature endothelial cells are rather added to the endothelium of preexisting blood capillaries than form new capillaries *de novo*. In many sections we failed to find endothelial progenitors beyond the limits of preexisting blood vessel wall. These cells did not leave the vessel and became stuck in the vessel wall, thus not creating any new vessels. Immature endothelial cells stuck in the vessels surrounded by thickened, blurred basement membrane formed a pattern of vascular loops with glomeruloid structure (Fig. 8).

Discussion

Characteristic pathological changes including thickening and accumulation of collagen fibrils in the

basement membrane of capillaries in the brain were enhanced in AD as described earlier [7,17]. Horssen et al. [15] demonstrated that collagen type XVIII (non fibrillar collagen) is accumulated in amyloid-laden vessels and classic senile plaques and β-amyloid might trigger cells in the vessel wall to produce this specific type of collagen. Collagen type XVIII is capable of inhibiting endothelial proliferation and angiogenesis.

Such ultrastructural alterations in brain capillaries are detected in various pathological conditions such as hypertension, diabetes, stroke, atherosclerosis, head injury, transient ischemia, or due to the effect of thrombogenic factors. All these conditions are associated with blood brain barrier discontinuity. The lack of correlation between the stage of the disease and the aberrations of cerebral capillaries suggests that the changes in microvasculature are not a consequence of AD pathology [19]. One may wonder, however, whether AD results from vascular damage or dysfunction. Endothelial damage, pathological evidence of disruption of blood brain barrier and β-amyloid deposition in brain vessels are almost universal in the advanced stages of AD. The blood vessel theory has been expanded to hypothesize that potential defects in the blood-brain barrier are a result of serious head injuries. Although neuronal cell death is the pathologic evidence of AD, it is difficult to clarify the primary mechanism resulting in this pathology.

The hypothesis that cerebral microvasculature is a key factor in the pathogenesis of AD has been suggested earlier [12,26,29]. Although there is no evidence showing functional impairment of blood brain barrier permeability or transient alteration of BBB integrity during aging, resistance to endogenous albumin transvasation by microvascular systems adjacent to amyloid deposits [36] and BBB leakage was observed in AD [13]. The vascular pathology has been described in AD but relatively little is known about the pathogenic mechanisms by which brain endothelial cells contribute to dementia and lesions in AD brains. According to the current concepts, the brain vascular system is continually modified in order to maintain adequate cerebral blood flow and brain perfusion, so normal laminar flow in brain capillary vessels becomes disturbed in regions where discontinuity of BBB and ultrastructural changes in capillaries occur [5,6].

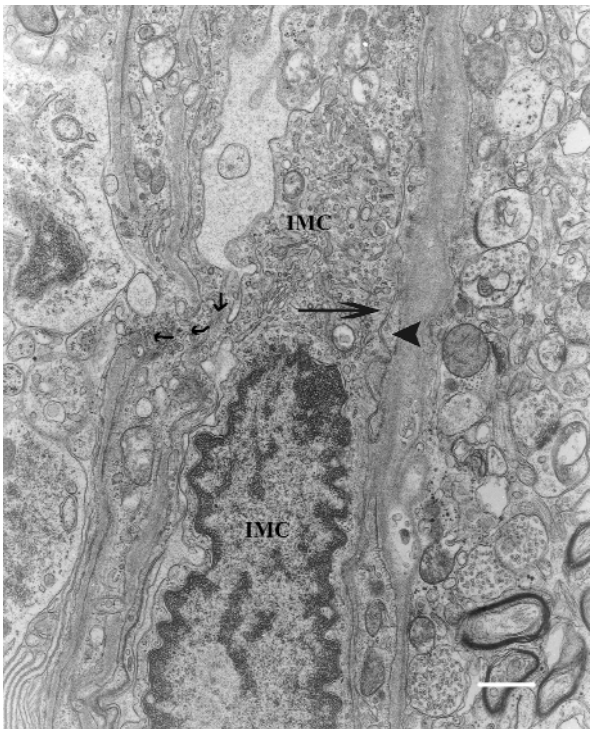

Fig. 6. The immature endothelial cell (IMC) leaving the vessel lumen (small arrows). The other one (big arrow) is lying on preexisting endothelium (arrowhead). Bar 2 μm

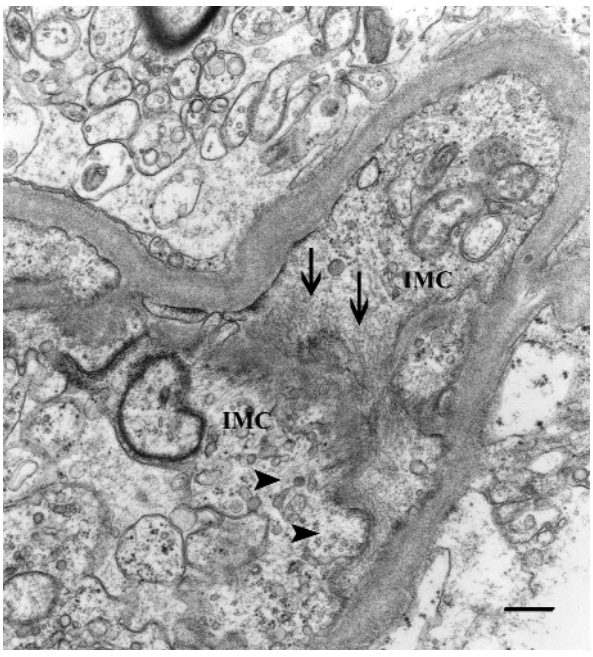

Fig. 7. A part of blood the vessel with stuck immature endothelial cell (IMC) characterized by cytoskeletal fibrils (arrowheads) and amyloid fibrils (arrows) in the cytoplasm. Bar 200 nm

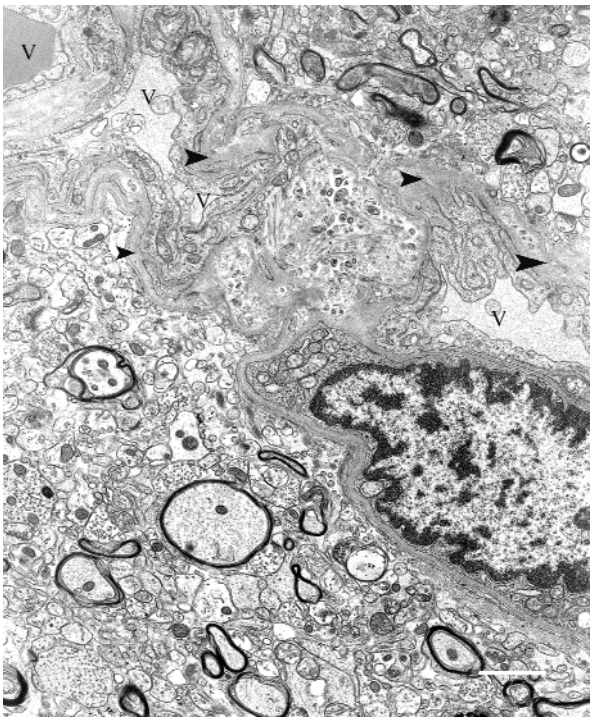

Fig. 8. A part of the vascular loop with glomeruloid structure and thickened basement membrane (arrowheads). V-vessel lumen. Bar 2 µm

What factors underlie disturbed blood flow in AD? The most interesting feature of analyzed material was new vessel formation. According to current concepts regarding new vessel formation, circulating cells are present in the peripheral blood that may contribute to neoangiogenesis in adults consistent with vasculogenesis. The ultrastructural features of these cells were described earlier by us in a surgical model of brain trauma [9] and confirmed by Flk-1 expression [10].

We can thus suggest that the pathological aspect of angiogenesis in AD is related to the perpetuating process of remodeling in most blood vessels, mediated by vascular progenitor cells and leading to decrease in the vessel lumen. Our ultrastructural findings are confirmed by earlier studies [20] that pointed to the absence of specific endothelial cell markers (CD34 and CD31) in AD-related degeneration.

The endothelial damage may be a functional disturbance rather than a result of cellular attachment and it seems that the process will continue as long as the injury is present. There is also a possibility that inflammatory factors that are elevated in AD [11] and destroy capillary walls are linked to a deficient repair process mediated by cytokines released by premature endothelial cells. Endothelial cytotoxicity induced by cytokines will increase blood brain barrier permeability [34].

The endothelial progenitor attachment to adult endothelium may result in blood vessel injury and induce formation of cytokines and other molecules. These molecules trigger not only a paracrine effect on the adjacent vessel wall but also an endocrine signal that is received by responsive bone marrow cells [28]. It was observed that with aging, and in the presence of risk factors, the progenitors originating from the marrow become incompetent, resulting in a loss of their capacity for repair of the vessel wall, which is in turn becomes dysfunctional. This was confirmed by our ultrastructural findings. If new vessel formation is an adaptative process in many pathologic situations associated with BBB discontinuity or hypoperfusion, endothelial precursors circulating in the blood and participating in that adaptation become an important element in AD. The mechanisms of repair and associated vascular growth are still poorly understood in the context of adaptation processes leading to neurodegenerative disorders.

As was shown in our studies, immature endothelial cells contain amyloid fibrils in cytoplasm. Some researchers discussed specific aspects of vascular dysfunction in AD related to aberrant transport of Aβ across the BBB and its role in the development of cerebral amyloidosis [37]. In these assumptions the BBB transport dysfunction for Aβ is a late event in AD, possibly resulting from disease-specific vascular disorder of brain endothelium associated with aberrant angiogenesis, cellular senescence and altered expression of a subset of genes. Our observations indicate that immature endothelial cells may be an important source of circulation-derived amyloid in the brain.

The consequence of immature endothelial cells should be appreciated more fully in both a pathobiological and therapeutic sense. While data regarding putative therapeutic potential of endothelial progenitors continue to mount, it is perhaps not improper to ask what is the biological context and time frame of their mobilization and activity. In this sense, features and impact of endothelial progenitors will probably change with better understanding of genetic,

phenotypic and microenvironmental characteristics of a given disease. The angiogenesis was initially only implicated in cancer, arthritis and psoriasis, but recently it has become evident that excessive, insufficient or abnormal angiogenesis contributes to the pathogenesis of many more disorders. The same may apply to immature endothelial cells.

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