

Invited Review

Cerebrovascular amyloidosis: Experimental analysis *in vitro* and *in vivo*

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Summary. With advancing age, the likelihood of β -amyloid deposition in the cerebral vasculature increases, particularly in individuals with Alzheimer's disease. The β -amyloid typically accumulates in the basal lamina of the arteriolar tunica media, and frequently extends into the adjacent neuropil. Cerebrovascular β -amyloid increases the risk of hemorrhagic stroke, and may also play a role in the pathogenesis of AD. Genetic variations have been identified that are causative or risk factors for cerebrovascular β -amyloid, including particular mutations in the genes for β -amyloid precursor protein, presenilins 1 and 2, and possibly cystatin C, as well as polymorphisms in apolipoprotein E. Cerebrovascular amyloidosis is now being studied in a variety of *in vitro* and *in vivo* models, including cultured vascular smooth muscle cells, transgenic mice, and aged animals such as nonhuman primates. Methods for delivering agents selectively to vascular amyloid in living subjects are now being developed, and these techniques are paving the way to the development of diagnostic tools and therapies for cerebrovascular amyloidosis.

Key words: Alzheimer's disease, β -amyloid, Cerebral amyloid angiopathy, Transgenic mice, Primate, Cystatin C, Stroke

Introduction

In cerebrovascular amyloidosis (CVA), amyloid is deposited in the walls of cerebral and meningeal blood vessels. Because it renders the vascular wall more susceptible to rupture, CVA increases the risk of intracerebral bleeding, and may be responsible for 15-20% of hemorrhagic stroke in the elderly (Vinters, 1987; Kase, 1994). CVA may also promote intracerebral hemorrhage in patients treated with thrombolytic agents, such as recombinant tissue type plasminogen activator

(Sloan et al., 1995). Although several different proteins can form the definitive β -pleated sheets of amyloid in the brain vasculature, the most common type of cerebrovascular amyloid derives from the β -amyloid peptide (A β), which also forms the cores of senile plaques in Alzheimer's disease (Wong et al., 1985; Van Duinen et al., 1987; Castaño and Frangione, 1988; Joachim et al., 1988; Prelli et al., 1988a). A β is a peptide of 39-43 amino acids that is derived from the β -amyloid precursor protein (BPP; Haass and Selkoe, 1993; Schenk et al., 1995). The form of A β in vascular β -amyloid differs somewhat from that in the brain parenchyma (Prelli et al., 1988a,b; Roher et al., 1993; Castaño et al., 1996), and also may be younger than plaque amyloid in the Alzheimer brain (Roher et al., 1993). There is evidence that vascular abnormalities may contribute to the genesis of senile plaques (Miyakawa et al., 1974, 1982; Glenner, 1979), a hypothesis that remains controversial (Kawai et al., 1990, 1992). While various conditions can promote CVA (Vinters, 1992), the most prominent risk factors for the disorder are increasing age (Tomonaga, 1981; Vinters and Gilbert, 1983; Esiri and Wilcock, 1986), Alzheimer's disease (Mandybur, 1975; Esiri and Wilcock, 1986; Yamada et al., 1987; Vinters, 1992; Ellis et al., 1996; Premkumar et al., 1996), and genetic influences (Ghisso et al., 1986; Luyendijk et al., 1986; Castaño and Frangione, 1988; Levy et al., 1989, 1990; Van Broeckhoven et al., 1990; Haan et al., 1991; Hendriks et al., 1992; Graffagnino et al., 1995; Greenberg et al., 1995; Petersen et al., 1995; Wattendorff et al., 1995; Bornebroek et al., 1996; Ólafsson et al., 1996; Premkumar et al., 1996; Vidal et al., 1996; Warzok et al., 1998). In Alzheimer's disease, there is a relationship between CVA and the degree of cerebral arteriosclerosis (Ellis et al., 1996).

Genetic influences on CVA

Genetics present a fortuitous window on the pathobiology of CVA. Older persons with one or two apolipoprotein E- ϵ 4 alleles, in addition to being predisposed to Alzheimer's disease, tend also to have a greater vascular β -amyloid load (Greenberg et al., 1995;

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Premkumar et al., 1996; Warzok et al., 1998). A β forms a complex with all three isoforms of apoE; however, in guinea pigs, the A β 1-40/apoE4 complex is transported across the BBB and sequestered by brain capillaries, whereas complexes of A β 1-40 with apoE2 and apoE3 are not (Martel et al., 1997).

CVA is caused by particular mutations in the genes coding for the precursors of several amyloidogenic proteins. Hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) arises from a missense point mutation at codon 693 (amino acid 22 within the A β region) of β PP (Levy et al., 1990; Haan et al., 1991; Hendriks et al., 1992; Bornebroek et al., 1996). The substitution of adenine for guanine at the "Dutch" locus recently has been found in Italian families with CVA and hemorrhagic stroke (Bugiani et al., 1998). Since the Dutch cases have a cytosine for guanine substitution at the same locus, it appears that the site, rather than the type, of mutation is key to the development of CVA. The Icelandic form of hereditary cerebral hemorrhage (HCHWA-I, or hereditary cystatin C amyloid angiopathy) is caused by a point mutation in the cystatin C gene (Ghiso et al., 1986; Castaño and Frangione, 1988; Levy et al., 1989; Graffagnino et al., 1995; Ólafsson et al., 1996), and meningocerebrovascular deposition of transthyretin-based amyloid results from a point mutation in the transthyretin gene (Petersen et al., 1995; Vidal et al., 1996). Although Icelandic cerebrovascular amyloid does not include A β , some β -amyloid deposits in both HCHWA-D and Alzheimer's disease contain non-mutant cystatin C (Maruyama et al., 1990; Vinters et al., 1990). The presence of Icelandic cerebrovascular amyloidosis is associated with abnormally low levels of cystatin C in

the cerebrospinal fluid (Löfberg et al., 1987; Shimode et al., 1991), possibly because the molecule is sequestered by the amyloidotic vessels (Löfberg et al., 1987). In Alzheimer patients A β levels in the cerebrospinal fluid also are reduced in the presence of abundant CVA (Pirttila et al., 1996).

It is not yet certain how the amino acid substitution at position 22 within A β results in the vascular accumulation of amyloid in HCHWA-D. The predominant form of A β in the vasculature of Dutch cases is A β 1-40 and its carboxy-terminal truncated derivatives (Castaño et al., 1996), confirming a strong relationship between CVA and A β 1-40 levels in tissue (Suzuki et al., 1994). *In vitro*, A β with the Dutch mutation more readily self-aggregates into fibrils (Wisniewski et al., 1991) of greater stability (Fraser et al., 1992) than does wild-type A β . Significantly, *in vitro* studies also indicate that the Dutch mutation augments the toxicity of A β 1-40 toward cultured smooth muscle cells (Davis and Van Nostrand, 1996). A link between A β aggregation and the toxicity of the peptide to vascular smooth muscle cells has recently been established. Blockade of aggregation via pretreatment with Congo Red prevents the smooth muscle cellular degeneration associated with exposure to the Dutch variant of A β 40 *in vitro* (Van Nostrand et al., 1998), suggesting that the assembly of amyloid aggregates on smooth muscle cells represents a key step in the pathogenesis of HCHWA-D. The fibrillar amyloid likely triggers a cascade of immune/inflammatory events that ultimately results in the characteristic loss of smooth muscle cells following amyloid deposition (Fig. 3).

Recently, CVA has been linked to two rare, familial forms of Alzheimer's disease involving mutations in

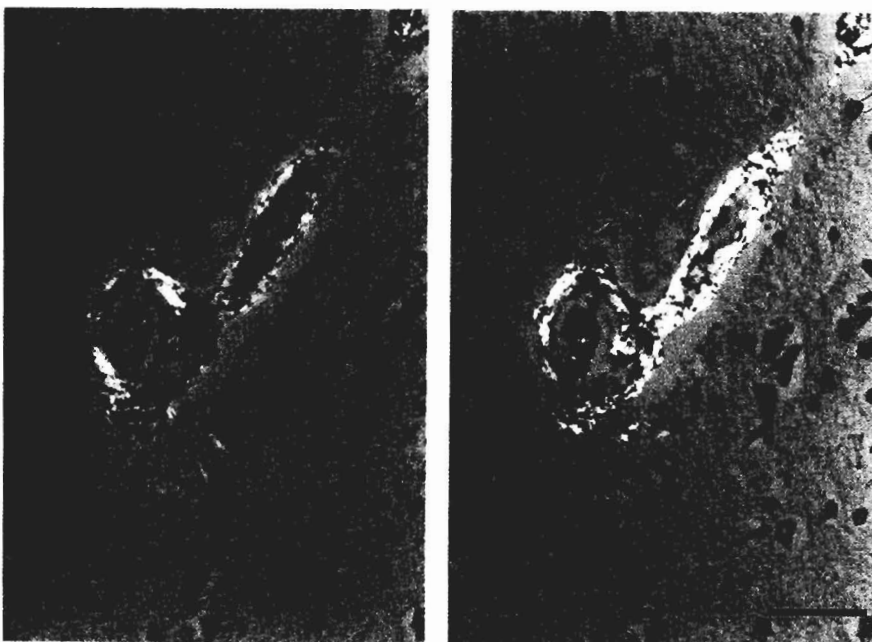


Fig. 1. Congophilic blood vessel in the neocortex of a person who had died of Alzheimer's disease. **A and B** show the same vessel under crossed polarizing filters; note the shift in birefringence when one polarizer is rotated slightly (arrows). Bar: 50 μ m.

presenilin genes. In a Volga German family, a mutation in presenilin-2 causes dementia with considerable CVA and relatively few senile plaques and neurofibrillary tangles (Nochlin et al., 1998). An unusual Finnish variant of Alzheimer's disease with deletion of exon 9 of presenilin-1 is characterized by spastic paraparesis followed by dementia, and a pathological signature of very large, diffuse-type β -amyloid plaques, neurofibrillary tangles and CVA (Crook et al., 1998). Thus, certain mutations in the presenilins can be added to the list of genetic causative or risk factors for CVA.

Anatomical and histological distribution of CVA

Amyloidotic cerebral blood vessels usually are located in the neocortex and adjacent leptomeninges; they are less common in the hippocampus, rare in the cerebellum and basal ganglia, and occur seldom, if ever, in the diencephalon and lower brainstem. Vessels in the white matter are infrequently involved. Arterioles are the most commonly affected vessel type, but amyloidotic venules and capillaries are sometimes seen (Mandybur, 1975). In any particular patient, CVA can be found in any lobe of the brain, and when vascular amyloid is abundant, more than one region is usually involved (Kase, 1994).

Cerebrovascular amyloidosis also is termed cerebral amyloid angiopathy or, because it frequently stains

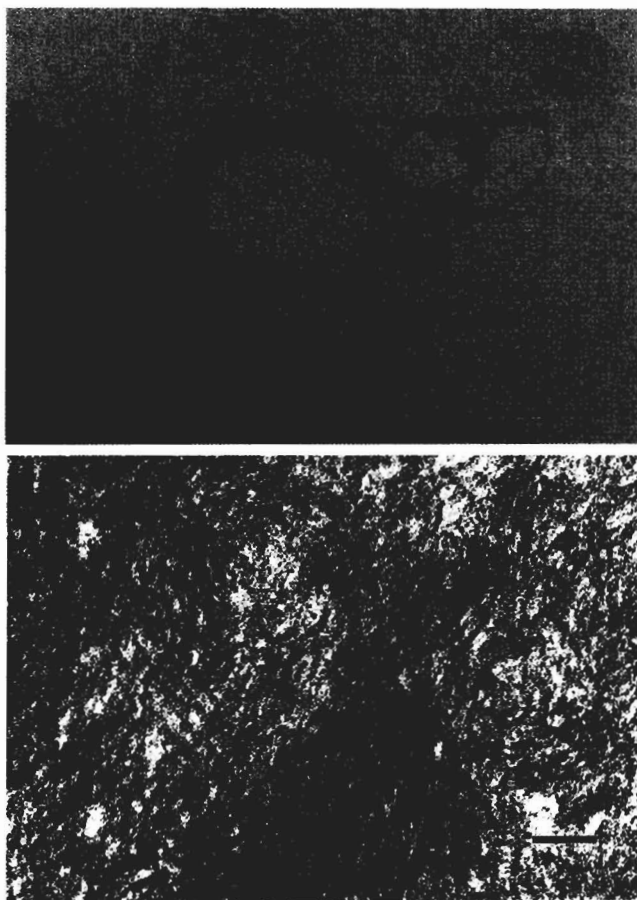


Fig. 2. A. Immunostained β -amyloid (arrow) in the *tunica media* of an arteriole in the neocortex of an aged (56-year old) Chimpanzee (*Pan troglodytes*) Bar: 25 μ m. B. Electron microscopic image of β -amyloid fibrils in the basal lamina (*tunica media*) of the aged chimpanzee. Bar: 100 nm.

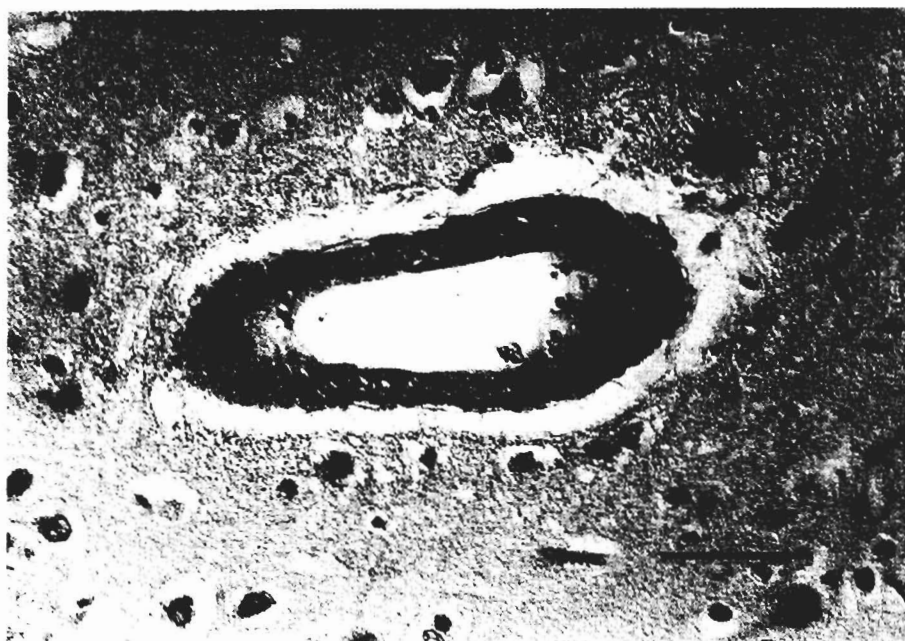


Fig. 3. Hematoxylin and eosin- (H&E) stained amyloidotic cortical arteriole in a case of Alzheimer's disease. The amyloid deposits are eosinophilic and smooth muscle cells are sparse, giving the vascular wall a hyaline appearance. Bar: 50 μ m.

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strongly with Congo Red (Fig. 1), *congophilic angiopathy*. CVA occurs primarily in the tunica media and tunica adventitia of arterioles. Within the vascular wall, the basal lamina appears to be a primary site of deposition (Miyakawa et al., 1974; Yamada et al., 1987; Perlmutter and Chui, 1990; Fig. 2); other components of the vessel are sometimes affected in CVA, such as the smooth muscle cells (Coria et al., 1989; Kawai et al., 1993; Wisniewski et al., 1994). Radiolabeled A β 1-40 binds selectively to amyloid deposits in the tunica media of sectioned arterioles *in vitro* (Maggio et al., 1992). When stained with hematoxylin and eosin, the amyloidotic arteriolar wall appears thickened and eosinophilic, with an amorphous, hyaline appearance; if amyloid is ample, the nuclei of smooth muscle cells within the tunica media are indistinct or absent (Fig. 3). In some cases, vascular amyloid deposits are coextensive with diffuse or plaque-like amyloid in the neuropil (Fig. 4). Ultrastructurally, cerebrovascular amyloid is composed of masses of fibrils of approximately 7-10 nm diameter (Fig. 2B).

In affected vessels, A β sometimes coexists with β PP (Tagliavini et al., 1990; Uno et al., 1996), and smooth muscle cells are implicated in the pathogenesis of CVA (Kawai et al., 1990; Wisniewski and Wiegel, 1994; Frackowiak et al., 1995; Wisniewski et al., 1995; Davis and Van Nostrand, 1996). The CSF contains soluble A β (Seubert et al., 1992), which, particularly in subarachnoid vessels, could be one source of vascular amyloid (Gherzi-Egea et al., 1996). However, other sources of A β must be considered in capillaries and venules of the neuroparenchyma, since these vessels lack smooth muscle cells. The bloodstream, pericytes, and cells within the neural parenchyma are three

possibilities. In humans with AD, the levels of soluble A β 1-40 in the brain correlate with the severity of CVA (Suzuki et al., 1994).

CVA is sometimes associated with cerebral vasculitis, and activated macrophages coexist with the amyloid deposits in granulomatous angiitis, sporadic CVA, and HCHWA-I (Wisniewski et al., 1996). In Alzheimer's disease, activated microglia colocalize with perivascular A β deposits as well (Uchihara et al., 1997). Nonsteroidal antiinflammatory drugs (NSAIDs) may reduce the risk of Alzheimer's disease (Rogers et al., 1996; Stewart et al., 1997), and have recently been shown to reduce activated microglia in the AD brain (Mackenzie and Munoz, 1998). It will be important to determine whether NSAIDs suppress microglial/pericytic involvement in CVA; animal models of the disorder will be useful for testing this hypothesis.

Given the rather uneven distribution of amyloid deposits within the walls of cerebral vessels, understanding the local factors that permit or promote the seeding and growth of aggregates is a significant issue. Bronfman et al. (1998) have begun to address this problem by demonstrating that laminin inhibits the aggregation of both wild-type A β 40 and the Dutch variant of A β 40. This process was blocked even when pre-aggregated fibrils were added to the solution, suggesting that laminin blocks the step at which additional fibrils/protofibrils are added to the existing aggregate. Laminin thus may be an important inhibitor of aggregation, especially at the level of the cerebral vessel.

A better understanding of the factors that influence amyloid aggregation in vessels also will have implications for treating CVA in the clinic. The

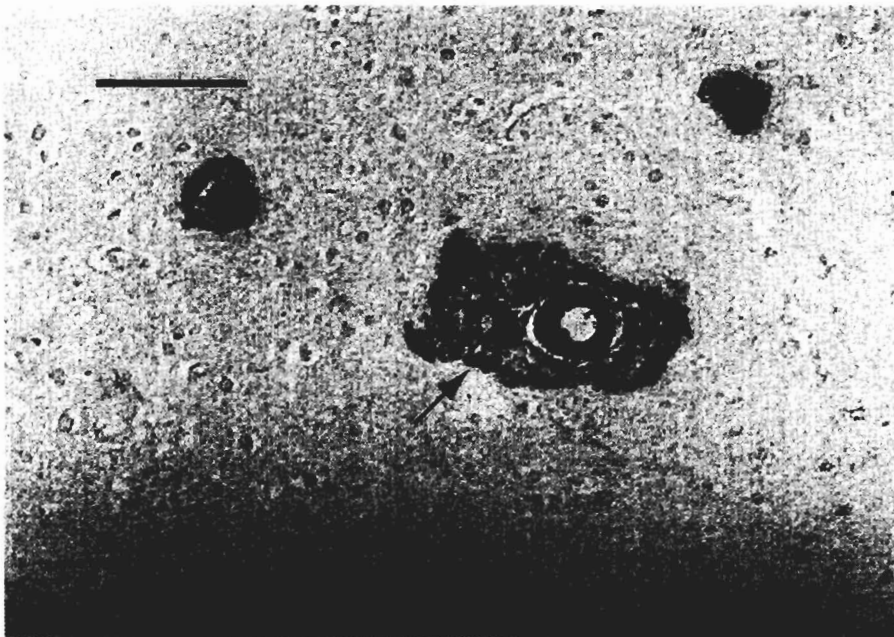


Fig. 4. β -amyloid-immunostained section of neocortex from a case of Alzheimer's disease with profuse CVA. Note the deposition of amyloid in the neural parenchyma surrounding the affected blood vessel. Bar: 100 μ m.

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progression of CVA from mild (asymptomatic) to severe (association with hemorrhage) represents an accumulation of amyloid in affected vessels rather than an increase in the number of vessels affected. Alonzo et al. (1998) studied this progression by evaluating the vasculature in postmortem brain samples. The severity of CVA in these cases was most closely correlated with an increase in the amount of A β 40 per vessel, while the proportion of vessels affected remained constant between mild and severe cases. Furthermore, increasing gene dosage of the ApoE4 allele from 0, 1 to 2 copies was associated with increased A β 40 load per vessel (Alonzo et al., 1998). Thus the progression from asymptomatic to advanced CVA is a result of the accumulation of amyloid in vessels that have been previously seeded with amyloid, and apolipoprotein E4 somehow promotes this process. The therapeutic implications of these findings are obvious; compounds that can inhibit the accretion of A β onto existing vascular amyloid deposits would be expected to prevent the development of amyloid-associated hemorrhagic stroke.

Animal models of CVA

A variety of nonhuman species naturally manifest CVA as they age, most notably nonhuman primates and dogs (see Walker, 1997 for review). While a small amount of immunoreactive vascular A β can be found in the brains of some BPP-transgenic mice (Fig. 5), it is not plentiful in most mouse models; however, one transgenic mouse expressing human BPP751 via a Thy-1 promoter (Sturchler-Pierrat et al., 1997) does develop significant CVA (Jucker et al., 1998). Full-length human BPP with

the Dutch mutation has been expressed in at least one line of transgenic mice, with no apparent cerebral β -amyloidosis in hemizygous animals up to 11 months of age (Greenberg et al., 1996). Furthermore, mice transgenic for the C-terminal 99 amino acids of human BPP have high blood levels of A β , yet they do not develop cerebral amyloidosis up to the age of 29 months (Fukuchi et al., 1996). Elevated circulating A β alone thus is not sufficient to induce CVA or senile plaques, at least in rodents. In aged nonhuman primates, however, cerebral β -amyloid can be labeled by circulating, synthetic A β (below).

Cerebrovascular β -amyloidosis is a common finding with age in many species of nonhuman primates, from prosimians to great apes (Walker, 1997). Some primate species are particularly prone to CVA, for example squirrel monkeys (Walker et al., 1990). In monkeys, CVA most frequently afflicts the neocortex. The globus pallidus, diencephalon, lower brainstem and cerebellum are unaffected. Between these two extremes lie a number of areas with varying degrees of CVA, including the amygdala, hippocampus, and the anterior neostriatum (the nucleus accumbens, in particular, sometimes has prominent vascular amyloid in monkeys; the caudate nucleus and putamen do not). Just as the *extent* of CVA varies among animals, so does the *pattern* of vascular involvement in susceptible regions. Within the neocortex as a whole, CVA often ranges from areas with severe CVA to areas with none at all. For example, both CVA and parenchymal amyloid are rare in the occipital lobe of rhesus monkeys, and relatively abundant in the anterior frontal and temporal lobes. The paucity of CVA in the occipital lobe of monkeys differs from AD cases with CVA and HCHWA-D cases, in which the occipital

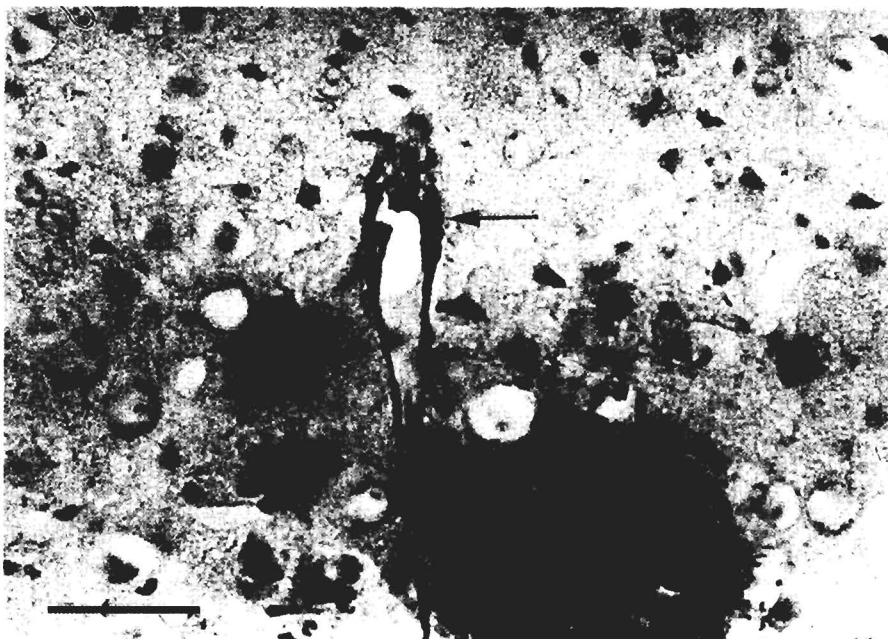


Fig. 5. Immunoreactive β -amyloid (arrow) in the wall of a cortical blood vessel from a mouse transgenic for human APP (APP695, K670N-M671L; Tg 2576 (Hsiao et al., 1996). CVA is rare in this transgenic model of cerebral β -amyloidosis. Note that the immunoreactivity appears to be within delimited structures, possibly cellular processes. Note also the parenchymal amyloid deposits in the region. Bar: 50 μ m.

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lobe can be substantially involved (Tomonaga, 1981; Wong et al., 1985; Vinters, 1987; Wattendorff et al., 1995).

Human vascular β -amyloid has relatively less of the longer, 42-amino acid form of the peptide than the shorter forms (Prelli et al., 1988a,b; Roher et al., 1993). In rhesus monkeys, arteriolar amyloid was labeled similarly with antibodies specific to the A β 40 and A β 42 carboxy-terminals, but many small cortical vessels were positive only for A β 42 (Gearing et al., 1996). A similar finding has been reported in cynomolgus monkeys (*Macaca fascicularis*) (Nakamura et al., 1995), and preliminary observations indicate a preponderance of A β 42 also in squirrel monkey capillaries, suggesting that capillaries and arterioles may contain different ratios of the C-terminal A β 40 and A β 42 molecules (see below). We should note in this regard that enzyme-linked immunosorbent assays (ELISA) indicate that immunohistochemistry-based quantitation may underestimate the levels of A β 42 in primate brain (Sawamura et al., 1997).

The squirrel monkey model of CVA

Squirrel monkeys (*Saimiri sciureus*) are small, New World monkeys that usually develop some degree of CVA by the age of about 15 years (Walker et al., 1990; Walker, 1993). Capillaries are more frequently affected in *Saimiri* than in rhesus monkeys and humans (Fig. 6). A β immunohistochemistry is the most sensitive method for detecting CVA in squirrel monkeys; conventional Thioflavin stains are somewhat less sensitive, and Congo Red birefringence marks only a small fraction of the vessels seen using immunohistochemistry. Capillaries in particular are seldom birefringent with Congo Red when viewed with cross-polarized light, and amyloid fibrils in

small vessels are difficult to detect by electron microscopy. Amyloid in large vessels is immunoreactive with C-terminal antibodies to both A β 40 and A β 42 in *Saimiri*, but capillaries contain mainly A β 42, (Walker, 1997) the form that predominates in diffuse parenchymal plaques. Capillary A β in squirrel monkeys thus may be soluble, or prefibrillar, in nature.

The preferential deposition of amyloid in the vasculature of squirrel monkeys might have a genetic basis. Our initial studies (Levy et al., 1995) found no evidence for a mutation either at codon 692 (Hendriks et al., 1992) or codon 693 (Levy et al., 1990) of β PP, and apoE in squirrel monkeys is essentially the same as that in other nonhuman primates (Gearing et al., 1994; Mufson et al., 1994; Poduri et al., 1994; Weisgraber et al., 1994; Calenda et al., 1995; Morelli et al., 1996). Because the amyloidogenic protein comprising CVA in squirrel monkeys is primarily A β , we were surprised to discover that these animals have a missense mutation in cystatin C at the Icelandic locus (Wei et al., 1996). An Icelandic-like mutation also has been implicated in a case of sporadic CVA in which both A β and cystatin C were deposited in the vascular walls (Graffagnino et al., 1995). Thus there may be link, as yet unexplained, between these two amyloidogenic proteins in some cases of human CVA. Mice transgenic for mutant human cystatin C might furnish clues to the role of this protein in both HCHWA-I and A β CVA.

Experimental analysis of cerebrovascular amyloidosis *in vitro* and *in vivo*

In vitro studies

A number of elegant studies have been conducted

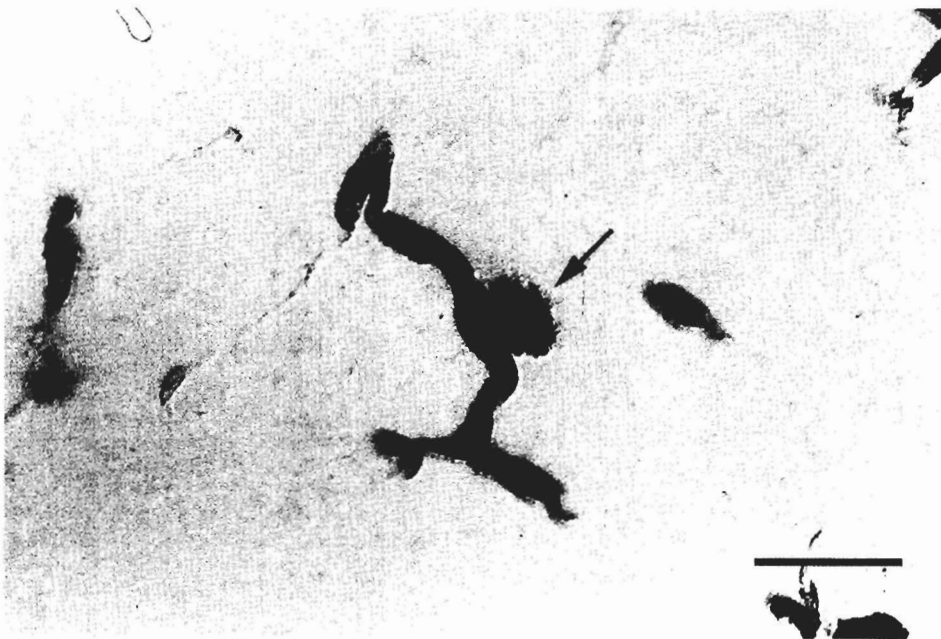


Fig. 6. Amyloidotic cortical capillary from an aged (14 years) squirrel monkey (*Saimiri sciureus*). Note the amyloid «bloom» extending from the vessel into the adjacent neuropil (arrow). Bar: 50 μ m.

using *in vitro* models of CVA, usually in cells or tissues from dogs. Canine amyloid deposits generally occur in the intercellular spaces of the arteriolar tunica media, similar to deposits in humans (Pauli and Luginbühl, 1971; Yamaguchi et al., 1992) and nonhuman primates (Cork and Walker, 1993; Uno et al., 1996), and supporting the hypothesized role of vascular smooth muscle cells in the production of arteriolar CVA (Kawai et al., 1993; Wisniewski and Wiegel, 1994; Frackowiak et al., 1995; Davis and Van Nostrand, 1996). Wisniewski et al. (1995) analyzed A β production in cultured smooth muscle cells, fibroblasts and endothelial cells from cerebral and peripheral blood vessels of young and aged dogs. Only myocytes from aged animals accumulated intracellular A β , and in some myocytes, electron microscopy showed that at least some of the A β was in fibrillar form. In living, organotypic cultures of leptomeninges and blood vessels from aged dogs, Prior et al. (1995) studied the binding of exogenous, fluorescein-labeled A β 1-40 to endogenous vascular amyloid. These researchers found that physiological concentrations of exogenous A β selectively bind to existing, extracellular deposits, and thereby contribute to the progression of CVA. In addition, the viability of cells in the vascular wall is diminished in many areas of β -amyloid deposition, possibly predisposing the vessels to rupture (Prior et al., 1996).

The pathophysiological effects of beta-amyloid have been investigated using bovine middle cerebral arteries. A β -induced endothelial damage was evidenced by increased vasoconstriction and reduced responsiveness to endothelium-dependent vasodilatory substances, i.e. acetylcholine and bradykinin (Price et al., 1997; Suo et al., 1997; Sutton et al., 1997; Thomas et al., 1997). Reactive oxygen species were implicated in the A β -induced cytotoxicity, since co-administration of SOD blocked the A β -induced increase in vasoconstriction. These findings suggest that alterations in vascular tone are the result of damage to the endothelial cells that is mediated by reactive oxygen species. More recently, an additional report has concluded that A β can increase vessel tone in an endothelium-independent fashion. Unlike A β -induced cytotoxicity, A β -induced vasoactivity is immediate, occurs in response to low doses of freshly solubilized peptide, and appears to be inversely related to the amyloidogenic potential of the A β peptides (Crawford et al., 1998). These effects were obtained in vessels lacking the endothelial layer. The investigators therefore concluded that the mechanism of A β -vasoactivity is distinct from that of A β -cytotoxicity. Although free radicals appear to modulate the vasoactive effects, the lack of requirement for endothelium suggests that the loss of the free radical balance (between NO and O $_2^-$) may be a secondary influence on A β enhancement of vasoconstriction. Although the mechanism of augmented vascular tone is not yet apparent, one could imagine a scenario in which elevated levels of A β induce a state of chronic, subclinical ischemia that could have long-term consequences for the pathogenesis of both

CVA and Alzheimer's disease.

The transendothelial transport of A β in nonhuman primates

There is a growing list of receptors that are capable of binding and/or transporting free A β as well as A β complexed to apolipoproteins J and E, including the receptor for advanced glycation end products (RAGE), gp330/megalin, scavenger receptor, and lipoprotein receptors (Martel et al., 1996; Zlokovic, 1996; Zlokovic et al., 1996; Poduslo et al., 1997). Circulating A β can be transported across the blood-brain barrier by a specific mechanism (Zlokovic et al., 1993; Maness et al., 1994; Poduslo et al., 1997). The permeability of the BBB to an A β /ApoJ complex is among the highest yet determined for a peptide or protein (Zlokovic et al., 1996). Furthermore, infusion of radiolabeled A β into the bloodstream of aged monkeys results in selective labeling of existing cerebrovascular amyloid deposits (Ghilardi et al., 1996; Mackic et al., 1998). The delivery of A β 1-40 to brain can be enhanced also by conjugating the peptide to a monoclonal antibody directed against the human insulin receptor (Wu et al., 1997). In contrast to other studies, Wu et al. (1997) found no evidence of transendothelial transport of circulating A β (not complexed to the insulin antibody) in normal adult rhesus monkeys. However, several factors, alone or in combination, could enhance the BBB transport and brain sequestration of A β in aged monkeys. For example, there may be augmented binding of A β to endogenous ligands for specific BBB transport systems (Zlokovic, 1996), reduced systemic clearance of the peptide, reduced peripheral and central metabolism, and greater concentration and retention of A β by pre-existing amyloid deposits (Mackic et al., 1998). Furthermore, species prone to developing CVA might have more vigorous (or more numerous) BBB transport systems for A β (Zlokovic et al., 1997). In any case, it is now clear that amyloid deposits in cerebral blood vessels can be labeled by ligands delivered through the bloodstream. The bulk of the data also indicate that naturally occurring A β in the bloodstream is one potential source of amyloid deposits in the senescent cerebral vasculature. Aged squirrel monkeys and dogs are presently the best available animal models for studying CVA *in vivo*, but we expect that transgenic mouse models of the disorder will soon become available to the research community as well.

Seeding of CVA in vivo

Finally, it is worth mentioning that studies with nonhuman primates suggest that CVA and senile plaques can be induced by intracerebral injection of Alzheimeric brain tissue. β -amyloid-containing human brain homogenates were injected into the brains of young marmosets (~2 years old), and the brains were analyzed 6-7 years later (Baker et al., 1993). CVA and senile plaques were found in several of the experimental

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animals, but not in age-matched controls. The results suggest that, like prion diseases (Prusiner, 1995), β -amyloid deposits in the neuroparenchyma and blood vessels can be induced by exogenously administered material. Although infusions of purified A β 1-40 into the primate brain have not produced plaques or CVA (Kowall et al., 1992; Podlisny et al., 1992), the elapsed time between infusion and sacrifice was only weeks to months, possibly too short for mature amyloid deposits to develop. In addition, the aged brain might be more vulnerable to exogenously administered amyloid (Yankner et al., 1998). Furthermore, A β 1-42 should promote *in vivo* fibrillization more effectively than does A β 1-40, and the inclusion of cofactors or "chaperones" for A β also might facilitate deposition (Afagh et al., 1996). The growing list of *in vitro* and animal models should accelerate hypothesis-testing on the cause, diagnosis and treatment of CVA in humans.

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