

Chapter 2

Transgenic Mouse Models for APP Processing and Alzheimer's Disease: Early and Late Defects

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Abstract: Transgenic mice with neuronal expression of human AD-mutant APP[V717I] in their brain recapitulate robustly the amyloid pathology as seen in Alzheimer's disease (AD) patients. The AD related pathological phenotype consisting of amyloid plaques and vascular amyloid pathology, develop progressively and relative late in ageing APP transgenic mice, between 10 and 15 months of age. In contrast to the late - and clinically irrelevant - amyloid plaque-pathology, the early cognitive defects and behavioural features are clinically more interesting. This review discusses the generation and in depth phenotypic characterization of both aspects of the APP[V717I] transgenic mice. Attention is focussed on the relation of biochemical data of the different APP fragments and amyloid peptides to the formation of the typical early defects and the late parenchymal and vascular amyloid depositions. The APP[V717I] transgenic mice are a perfect model to characterize and investigate early biochemical and cognitive aspects and a potential resource to define pathological interactions of different factors known to be involved in AD. Finally, any therapeutic intervention can be directly tested and explored in these transgenic mice as excellent pre-clinical models

Key words: Transgenic mice, Alzheimer's disease (AD), amyloid-plaque-pathology.

1. INTRODUCTION: GENETICS AND PATHOLOGICAL FEATURES OF ALZHEIMER'S DISEASE

Alzheimer patients are characterized by mostly atypical clinical features during life and by very typical pathological lesions in their brain, observed

post-mortem: extra-cellular amyloid deposited as parenchymal plaques and vascular angiopathy, in addition to intra-cellular neurofibrillary tangles. Because of the ill-defined and largely unknown relations between the clinical and pathological problems on the one hand, and between both pathological lesions on the other hand, this chapter will first concentrate on the brain pathology and its robust recapitulation in transgenic mouse brain. Those late defects will then be related and traced back to early defects, mainly referring to behavioral and cognitive aspects that are obvious even at the "pre-amyloid" stage of the disease in APP transgenic mice.

Inherited familial forms of AD are very rare (<1% of all cases) and mainly caused by mutant Presenilin genes. Most AD cases are sporadic of unknown ethiology (for reviews see St. George-Hyslop, 2000; Selkoe, 2001), but all AD cases comprise by definition the pathological combination of amyloid plaques and intra-neuronal tau-aggregates (Braak and Braak, 1991; Delacourte and Buée, 2000). Independent of their genetic make-up or epi-genetic history, all familial and sporadic AD patients present with the same pathological lesions in their brain at autopsy, *i.e.* extracellular amyloid senile plaques and intracellular neurofibrillary tangles, both concentrated in the hippocampus and the cerebral cortex. This is the pathological definition of this neurodegenerative disorder, brought "to light" almost a century ago by silver impregnation of brain sections (Alzheimer, 1906). Practically all AD-patients have also amyloid deposits in the wall of small blood vessels in the brain, although comparable cerebral amyloid angiopathy (CAA) is also evident in about a third of all elderly people. In this chapter we will concentrate on the amyloid pathology in brain parenchym and vasculature and on its successful recapitulation in the brain of APP[V717I] transgenic mice - and we will explore how the amyloid hypothesis is tested and extended towards the early defects. It must be remembered that knowledge of these early defects, not only in clinical and cognitive terms, but especially in functional and molecular detail, is essential for the development of early detection and treatment of this devastating diseases, *i.e.* before irreversible brain damage has occurred.

1.1 Amyloid plaques in brain

The types of amyloid deposits that are evident in the parenchym of the human brain are named as diffuse and as neuritic or senile plaques. The latter are very typical and diagnostic for AD. They consist predominantly as deposits with a central core of amyloid that is up to 100 μm across and are surrounded by abnormal neuronal processes originating from neighboring cells. These swollen and dystrophic neurites are distended and contain a variety of degenerating cellular organelles, mitochondria and lysosomes,

besides being immuno-reactive for many synaptic and cytoskeletal markers. Interspersed among the neurites are processes and occasionally also cell-bodies of activated microglia and of activated astrocytes. This is taken as evidence for an ongoing inflammatory reaction in and surrounding the plaques that is attracting more and more attention in terms of its negative pathological involvement and as potential therapeutic target.

1.2 Cerebral amyloid angiopathy (CAA)

The abluminal deposition of amyloid peptides in cerebral vessel walls is inherent and a diagnostic element of the AD pathology. CAA occurs sporadically and is observed in a third of all elderly people over 60. The vascular amyloid is deposited most commonly in meningeal and cortical arteries and arterioles, and less frequently in veins and capillaries. Vascular amyloid disturbs the smooth muscle cells and the basal lamina of the vessel walls, causing degeneration and weakening of the vessel walls and provoking small and large aneurysms. These lesions have been estimated to be responsible for 15% of all hemorrhagic strokes in elderly people. The CAA as an essential pathological feature in AD patients might constitute a direct link to the closely related and clinically difficult to distinguish entity of vascular dementia, the etiology of which is unknown but involves defects in the vascular wall. In daily clinical practice, the difficult differential diagnosis of AD and vascular dementia has led to the assumption that these are two extremes of a similar pathology, with a mixed type of dementia in between that is hard or even impossible to define precisely.

1.3 Neurofibrillary Tangles

Neurofibrillary tangles (NFT) consist mainly of protein tau and typify not only AD but many neurodegenerative diseases, *e.g.* fronto-temporal dementia (FTD), corticobasal degeneration, Pick's disease, ... collectively termed tauopathies (Delacourte and Buée, 2000). Primary tauopathies are caused by diverse mutations in the tau gene and named fronto-temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) (Heutink, 2000; Delacourte and Buée, 2000; Ingram and Spillantini, 2002). AD is not a primary, but by far the most prevalent tauopathy, however it is not caused by mutations in the tau gene.

Aggregated protein tau is invariably hyper-phosphorylated supporting the assumption that hyper-phosphorylation is inherent to the mechanism of tangle formation. In addition, hyper-phosphorylated protein tau readily forms paired helical filaments *in vitro* while de-phosphorylation decomposes tau-filaments into soluble tau-monomers. Conversely, it cannot be excluded

that in diseased human brain, neuronal protein tau forms aggregates that subsequently become hyper-phosphorylated and thereby stabilized, causing or contributing to their neurotoxicity by disturbing axonal transport (for review see Terwel *et al.*, 2002).

2. MAJOR QUESTIONS IN AD

Three phases are recognized in the clinical condition of AD patients: (i) mild cognitive impairment (MCI) with minor short-term (recent) memory problems, progressing into (ii) obvious and then massive deficits in learning and memory with important repercussions in personality, emotional state and character (intermediate clinical stages), rapidly worsening into (iii) deep dementia with associated “brain atrophy” (late clinical phase).

The first and major clinical feature or symptom that is observed but not typical for AD patients is the inability to learn and remember new facts. In the absence of objective biochemical or molecular methods to diagnose AD early and reliably, the clinical diagnosis is uncertain and heavily dependent on the actual stage, evolving from “probable” to “possible” to practically “definite” AD only in the late clinical stages. It must be remembered that definite diagnosis can only be obtained post-mortem, by pathological examination of brain sections after silver staining (*cf.* A. Alzheimer, 1906).

It is evident that objective and early diagnosis is absolutely needed, not only to solve the social and clinico-medical problems, but even more so to allow the pharmacological and/or other developments of effective therapy. The many problems encountered in this respect are beyond the scope of this chapter, but readers should be aware of their utmost importance.

The major problem in AD remains to decipher what is cause, correlation and consequence of the disease, despite - or perhaps due to - the multiplicity of molecular and biochemical defects and brain lesions that have been and are being reported. Unanswered questions remain whether dementia and neurodegeneration in patients are equal to loss of neurons or only of synapses, or whether only a functional disturbance of synaptic transmission is at stake. Other important issues in the overall pathogenic process – which emerge in many hypotheses – are the apoptosis of neurons and loss of synapses and the malfunction of the glutamate neurotransmitter system, resulting in excitotoxicity.

Here, we will concentrate on transgenic mice that recapitulate robustly the amyloid related neuro-pathology as seen in AD patients. This is a major contribution to understand the biochemical and physiological aspects of amyloid precursor protein (APP), of α - and β -secretase and of presenilin in the complex context of the brain. It is generally believed that mishaps in

processing of APP, in which presenilin is intimately involved (De Strooper *et al.*, 1998) will eventually lead to amyloid peptide production as the central problem in AD (Hardy, 1997; Selkoe, 2001).

Many aspects of the pathogenesis and the role of amyloid plaques and of cerebral amyloid angiopathy in AD remain for a large part unclear. The fact that senile plaques and vascular amyloid are diagnostic in all AD patients and that they consist mainly of amyloid peptides, has been taken to suggest that they are essential and central in the overall process of "amyloidogenesis" in AD. All other and non-specific clinical and pathological features of AD are thought to be secondary to or caused by amyloid peptides, *i.e.* synaptic loss, gliosis, neuronal loss, brain-shrinkage, and even neurofibrillary tangle formation. Nevertheless, the in-depth characterization of APP transgenic mice, and of multiple transgenic derived strains, have yielded strong evidence that the amyloid pathology does not *per se* involve physical deposition, but that the concept of the amyloid cascade must incorporate the early stages where the amyloid peptides are present as dimers, oligomers and small aggregates. How these cause the early defects in synaptic transmission or synapse loss, probably underlying MCI and early stage AD, constitutes the evident challenge for this research-field.

3. A ROBUST MODEL FOR AMYLOID PATHOLOGY: APP[V717I] TRANSGENIC MICE

We generated and have characterized in depth the transgenic mouse strain named APP-London or APP[V717I] that shows all the robust and reproducible features of the late amyloid pathology as observed in the brain of AD patients: starting with diffuse amyloid plaques that evolve with ageing into numerous thioflavine-S-positive senile plaques, followed by extensive cerebral amyloid angiopathy. This transgenic mouse strain was obtained by overexpression of the London-mutant of human amyloid precursor protein (APP) *i.e.* APP[V717I], carrying the mutation just Down'sstream of the γ -secretase cleavage site. This human gene was incorporated into a construct based on the neuron-specific elements of the murine thy-1 gene promoter (Moechars *et al.*, 1996, 1999). In the brain of heterozygous APP[V717I] transgenic mice, the level of over-expression of human APP is about 2 to 3 times higher than that of endogenous murine APP. Significant for the operational aspects of this model, the expression of human APP is restricted exclusively to the neurons of the central nervous system, as demonstrated by *in situ* hybridization and by immuno-histochemistry (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000) and the expression is moreover appreciable only in

the second or third week after birth, thereby eliminating any interference with the development of the brain.

3.1 Occurrence and progression of diffuse and senile amyloid plaques

At about one year of age, the APP[V717I] transgenic mice begin to develop pathological features that are strikingly similar to those observed in the brain of AD patients. From that point onwards, the APP[V717I] transgenic mice develop diffuse and neuritic plaques with a morphology that is characteristic for and practically identical to those observed post-mortem at autopsy in human brain. The senile or neuritic plaques contain a core of amyloid that stains intensely with thioflavine-S (Figure 1) and with Congo red, surrounded by numerous dystrophic neurites. Ultrastructurally, the core consist of bundled amyloid fibers that are 8 to 10 nm in diameter that are immuno-stained with practically all antibodies directed against amyloid peptides of either 40 or 42 amino acids, *i.e.* A β 40 and A β 42. Diffuse plaques in APP[V717I] transgenic mice are detected immuno-histochemically with a pan-A β antibody (Figure 2) as well as with antibodies directed against A β 40 and A β 42. Similar to AD patients, these diffuse plaques are quantitatively more important - by about a factor of 10 - than the senile, thioflavine-S-positive plaques in ageing APP[V717I] transgenic mice. Amyloid plaques develop first in the subiculum and spread to other regions of the hippocampus and the cerebral cortex. With ageing they continue to increase in numbers, up to about 20-24 months of age of the transgenic mice, and remain preponderantly in the hippocampus and neocortex, but are also evident in thalamus and other brain regions, but are never observed in the cerebellum (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000; Dewachter *et al.*, 2002). These qualitative and quantitative features of the amyloid plaques, *i.e.* type, evolution and regional distribution approach very closely what is known of the amyloid pathology in AD patients, as far as it can be progressively traced by post-mortem analysis of occasional cases, dying from accidental or unrelated causes.

3.2 Cerebral amyloid angiopathy

Besides and in addition to the amyloid plaques in the brain parenchym, the older APP[V717I] transgenic mice progressively develop very abundant, thioflavine-S-positive amyloid deposits in their cerebral blood vessels. The phenomenon of cerebral amyloid angiopathy (CAA) is at first evident in the

leptomeningeal pial blood-vessels, and further develops in cortical, thalamic, and hippocampal vessels (Figure 3). The accumulation of amyloid in blood-vessels is observed some few months later than the amyloid depositions in the brain parenchyma, *i.e.* in APP[V717I] transgenic mice that are older than 15 months of age. This indicates that they form later than the amyloid plaques and, similar to AD patients, show variation in individual APP[V717I] transgenic mice, both in distribution and extent.

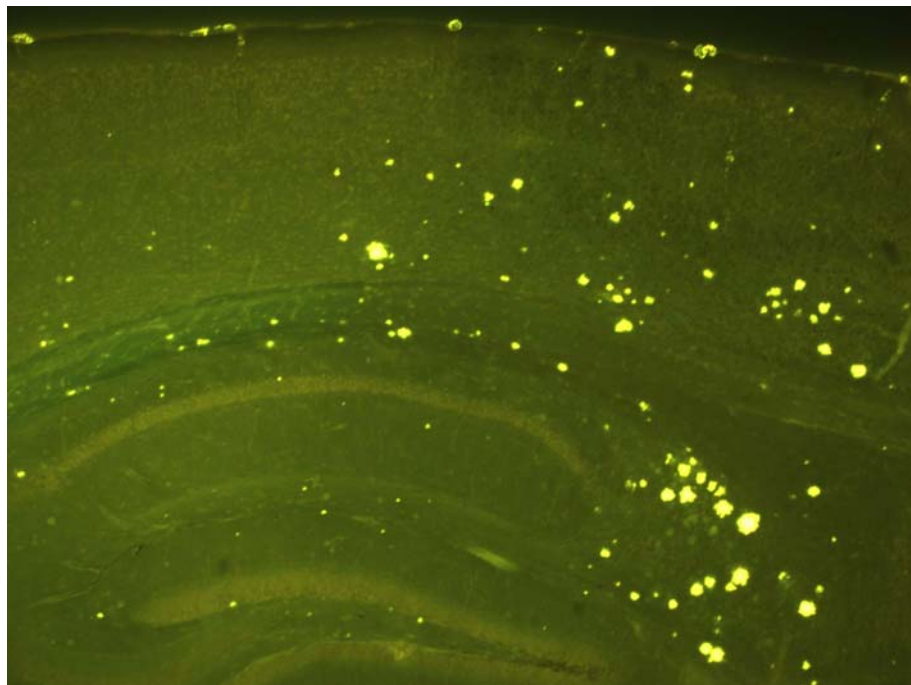


Figure 1. Thioflavine-S staining of typical neuritic plaques in the subiculum, hippocampus and the neocortex of a 22 months old APP[V717I] mouse. Bar=300 μ m

A minor difference is that neither diffuse nor senile plaques, nor vascular amyloid deposits were ever observed in the cerebellum of APP[V717I] transgenic mice. This is due to the low expression of the human transgene in cerebellum relative to forebrain. This is an intrinsic characteristic of the mouse *thyl* gene promoter construct, but since in AD patients cerebellar pathology is also not well developed, this aspect is in fact a bonus of the APP[V717I] transgenic model.

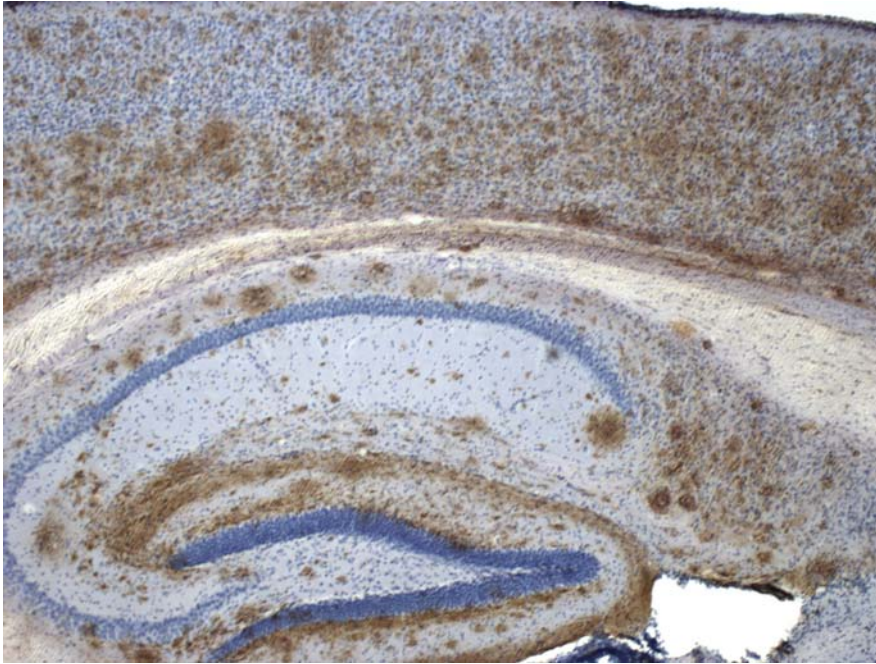


Figure 2. Immunohistochemical detection of diffuse and senile plaques with pan-AB in a 22 months old APP[V717I] transgenic mouse. Bar=300 μ m

The staining with thioflavine-S reveals the most severely affected arteries to exhibit amyloid-based fluorescence in patterns of concentric rings, while less affected vessels show focal abluminal accumulations similar to human CAA. Arteries are more affected than veins that show only small focal accumulations. Capillaries are rarely affected in the APP[V717I] mouse brain but some capillaries have been observed with amyloid depots penetrating into the neuropil, resembling dyschoric amyloid in AD patients. Very similar to human CAA, the large arteries at the base of the transgenic mouse brain constituting the circle of Willis, nor the extra cranial blood vessels were affected (Van Dorpe *et al.*, 2000). Vessels with important amyloid deposition show loss of smooth muscle cells, while rarely the internal elastic lamina damaged. The vessel wall damage leads to dilation and aneurysms, again as in human patients, but hemorrhage was not evident in brain of APP[V717I] transgenic mice. Moreover, the cerebral blood flow was measured by laser-Doppler flowmetry and proved unaffected even in very old APP[V717I] transgenic mice.

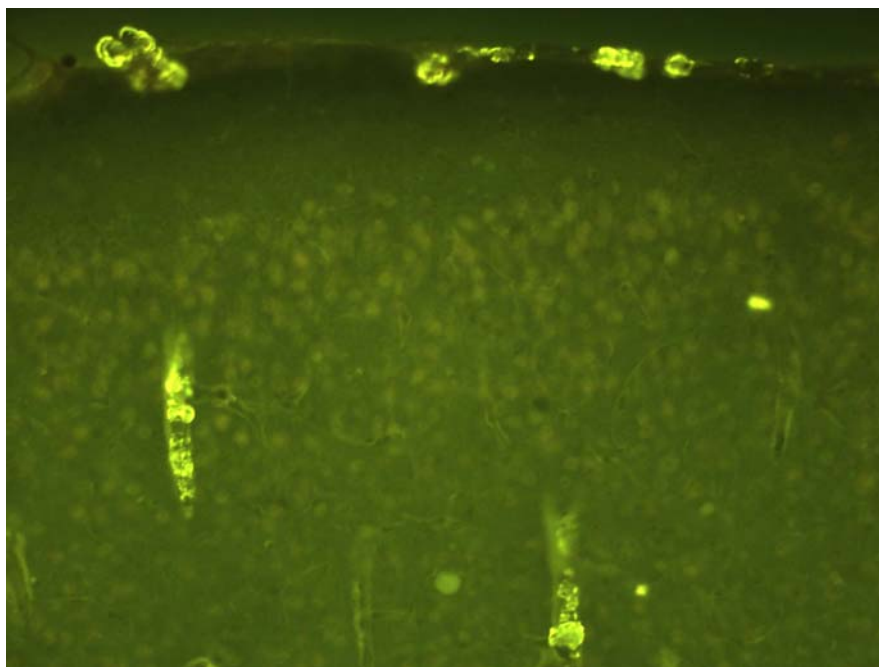


Figure 3. Cerebral amyloid angiopathy (CAA) in a 22 months old APP[V717I] transgenic mouse. At this age, thioflavine-S-positive amyloid deposits are abundantly present in the leptomeningeal pial (arrows) and cortical (arrowhead) bloodvessel. Bar=100 μ m

3.3 Ageing and amyloid pathology, a paradox of pathology versus biochemistry

In APP[V717I] transgenic mice, both vascular and plaque amyloid load progressively increased with age. Quantitatively, amyloid plaque pathology begins to develop in the 10-12 months age window, while vascular pathology forms not before the age of 15 months. Both types of amyloid pathology progress further and continue to increase quantitatively during the 2nd year of life of these mice. At all age-points analyzed, the thioflavine-S and immuno-reactive amyloid plaque load correlated closely with the number of vessels containing vascular amyloid (Van Dorpe *et al.*, 2000). In APP[V717I] transgenic mice 2 years of age, more than 90% of the leptomeningeal arterioles over the cerebrum were abundantly loaded with amyloid deposits. At that age, the subiculum of these old mice is literally inundated with diffuse and senile plaques, similar to some cortical regions.

In humans, ageing remains the most important, but least understood parameter or risk-factor for dementia and for AD. The APP[V717I] transgenic mice were therefore compared at ages of 3 months, 6-9 months

and 15 months, to measure APP and its different products of proteolytic processing, *i.e.* the membrane bound precursor (APP_m), the soluble and plaque-associated amyloid peptides A β 40 and A β 42, the secreted ectodomain of APP processed by α - or β -secretase (APP_s α , APP_s β) and the C-terminal transmembrane and cytoplasmatic domain resulting from β -secretase cleavage, referred to as " β -C-stubs" (Dewachter *et al.*, 2000). The comparative analysis revealed that ageing did not appreciably affect the normalized levels of either the α -secretase cleaved ectodomain nor the residual β -secretase cleaved C-terminal stubs of APP. Between the age of 3 to 15 months, the most pronounced effect concerned the dramatic and expected increase in insoluble amyloid peptides, A β 40 and A β 42, as well as an increased A β 42/40 ratio.

Ageing of the APP[V717I] transgenic mice caused progressively more aggregation of the amyloid peptides in the physical form of amyloid plaques, due to increased levels of soluble amyloid peptides, especially of the least soluble A β 42. This phenomenon did however, never occur before the age of 10-12 months in the APP[V717I] transgenic mice, as observed in unrelated APP transgenic mouse models in which A β also increased with age (Hsiao *et al.*, 1996; Johnson-Wood *et al.*, 1997). Measurements of the mRNA coding for endogenous murine APP and for human transgene APP[V717I] increased with age in the brain of transgenic mice (Moechars *et al.*, 1999). Membrane-bound APP protein increased in close parallel with mRNA levels, with levels reaching 122% and 169% respectively at 6-9 months and 15 months (Dewachter *et al.*, 2000).

The combined data demonstrate that in brain of the APP[V717I] transgenic mice, ageing *per se* did not markedly affect the normal processing of APP as mediated by α - and β -secretase. Therefore, the marked increase in both soluble and plaque-associated amyloid peptides, essentially situated at the age of 12 months, was not a direct consequence of a disturbed or tilted balance in the two competing proteolytic events that govern APP metabolism, *i.e.* the non-amyloidogenic pathway by ADAM10 as α -secretase (Postina *et al.*, 2004; *see also* Chapter 6) and the amyloidogenic pathway mediated by the combined actions of β - and γ -secretases (reviewed by Dewachter and Van Leuven, 2002). Whereas amyloid deposition is unequivocally further increasing with age, the production of amyloid peptides is not increasing! This apparent paradox between pathology and biochemistry points to another mechanism, *i.e.* that degradation and/or clearance of the amyloid peptides must be impaired in the APP[V717I] transgenic mice - and by extrapolation, also in the brain of AD patients (see Chapter 7). This conclusion implies, among other factors, an operational mechanism of liquid drainage from the brain into the CSF, functionally similar to the lymphatic system in peripheral organs (Weller and Nicoll,

2003) which thereby directly connects the amyloid depositions in parenchym and in the peri-vascular spaces, *i.e.* the amyloid plaques and the CAA pathology, as discussed in the following section.

3.4 Pathogenesis of cerebral amyloid angiopathy

A long-standing debate concerns the origin of the vascular amyloid depositions. In human and mouse brain, as well as in other tissues, endogenous APP is ubiquitously expressed by many cell types, including these of the vasculature. Some proposed that A β in the vessel walls would derive from vascular smooth muscle cells or pericytes since pial vessels are most often affected, implying local production as potential source of A β . This hypothesis failed, however, to explain the specific neuroanatomical pattern of cerebral amyloid angiopathy and its exclusive localization in intracranial vessels.

We demonstrated by Western blotting that blood plasma of aged APP[V717I] transgenic mice did not contain detectable levels of amyloid peptides (*i.e.* lower than picogram-levels) as opposed to CSF that contains A β in the range of 10-20 ng/ml (Van Dorpe *et al.*, 2000). This finding, combined with the strong evidence for the exclusive neuronal expression of the APP transgene in this mouse strain (Moechars *et al.*, 1999) and the abluminal deposition of vascular amyloid (Van Dorpe *et al.*, 2000), unequivocally demonstrated that amyloid peptides originating exclusively from neurons within the brain are the direct cause of amyloid plaques in the brain parenchym as well as of the CAA.

CAA appears in vessels that do not express the transgene, but evidently requires and implies a means of transport of amyloid peptides to the deposition sites. This problem is less evident or obvious in the formation of senile plaques. Amyloid peptides are present in the CSF of normal and AD individuals and drainage pathways must be involved along the perivascular space surrounding intracortical and leptomeningeal arteries. These channels eventually connect with nasal lymphatics draining to the cervical lymph nodes. The suggestion that significant amounts of A β drain along this pathway in humans (Weller *et al.*, 1998) is fully confirmed by the objective analysis of all data in the APP[V717I] transgenic mice (Van Dorpe *et al.*, 2000). Recently, in AD patients the hypothesis of the neuronal origin of A β and the drainage of A β via the interstitial fluid from the central nervous system to capillary walls is confirmed in AD patients (Roher *et al.*, 2003; Attems *et al.*, 2004).

3.5 A β 42 is the primary cause of amyloid deposition in parenchyma as well as in blood vessels

In brain of AD patients, the ratio of A β 42 to A β 40 is higher in plaques than in the vascular amyloid deposits, which is recapitulated in the APP[V717I] transgenic mice that also have much higher A β 42/A β 40 ratios in the amyloid plaques than in the vascular amyloid (Van Dorpe *et al.*, 2000). The purely neuronal origin of the amyloid peptides then allows us to define their path and their fate.

After secretion by neuronal cells (by as yet unknown mechanisms) the level of the amyloid peptides in the intercellular and interstitial spaces increases to reach levels at which the least soluble A β 42 peptides begin to aggregate into oligomers (*see also* Chapter 1). These form the nuclei for further aggregation in which also A β 40 peptides participate, as well as less abundant A β 38 and eventual other species. These aggregates develop into diffuse and progress later into senile plaques by accruing more amyloid and other insoluble proteins that are either trapped or otherwise become lodged into these plaques, that can reach diameters of up to 1 mm.

At the same time, excess peptides are being drained by the perivascular route into the CSF, eventually taking also some of the smaller aggregates to flow along and become trapped in the perivascular spaces. These form the nuclei of the CAA, whereby evidently the more soluble A β 40 peptides diffuse faster and further than the least soluble A β 42 peptides. A gradient of A β 42 thereby must form *de facto*, with highest levels in the neurons and decreasing towards the CSF - explaining also why in AD patients the A β 42 concentration in CSF actually decreases with the progression of the pathology (Blennow *et al.*, 2003).

Hence, similar to senile plaque formation, the A β 42 is first to become deposited in the vessel walls (nucleation) while the more soluble A β 40 is subsequently entrapped (growth). This is further supported by the earlier and increased formation of senile plaques and of cerebral amyloid angiopathy in double transgenic APP[V717I] x PS1[A246E] mice, obtained by crossing the respective single transgenic mice. In these double transgenic mice, the extra incorporation of the human mutant PS1 transgene caused a selective increase in production of A β 42 over A β 40 (Dewachter *et al.*, 2000).

More recent evidence in BACE x APP[V717I] double transgenic mice add further weight to this hypothesis, demonstrating that N-terminal truncated forms of the A β peptides that are even less soluble than A β 42, actually increase amyloid deposition in brain parenchyma while reducing CAA in the vessel walls (Willem *et al.*, 2004). The fact that N-terminal truncated A β x-40/42 aggregate faster and more easily than the full-length counterparts (Liu *et al.*, 2002) further supports the hypothesis as well as the

negative correlation between plaque and CAA pathology in human AD brain (Tian *et al.*, 2003).

Although APP[V717I] transgenic mice express APP at higher levels than in human brain, the striking similarity to human cerebral amyloid angiopathy is more than a strong suggestion that similar mechanisms cause the vascular amyloid deposition in patients and transgenic mice, alike.

4. EARLY COGNITIVE AND BEHAVIOURAL DEFECTS OF APP[V717I] TRANSGENIC MICE

Besides the specified and characteristic pathological defects caused by insoluble A β 40 and A β 42, the APP[V717I] transgenic mice additionally display early phenotypic behavioural and cognitive impairments (Moechars *et al.*, 1999). With the revival of the hypothesis of "synaptic deficit" in AD (Selkoe, 2003) these early defects were and will be more and more interesting as typical characteristics of a mildly impaired cognitive phenotype and as read-out to define effects of genetic or epigenetic factors and treatment (Dewachter *et al.*, 2002; Dewachter and Van Leuven, 2002).

As opposed to plaque formation that develops after 10-12 months of age, disturbed behaviour and cognitive defects occur at an age as early as 3-6 months. Profound disturbances in behaviour are evident as hyperactivity, anxiety, aggression and neophobia. Some of these obvious and typical characteristics were observed in APP[V717I] transgenic mice as young as 8 weeks and came progressively more evident with age. Cognition, spatial learning and memory as estimated by the Morris water maze paradigm, by novel object recognition and by contextual d-fear conditioning are significantly impaired compared to age and sex-matched non-transgenic mice with the same genetic background (Moechars *et al.*, 1998, 1999; Dewachter *et al.*, 2000; 2002). In addition, a progressive decay of long term potentiation (LTP), an accepted parameter or even model for synaptic plasticity, was evident long before any plaques were formed in the brain parenchym. Intriguingly, the early behavioural and cognitive defects were also observed to a large extent in transgenic mice that overexpressed the human APP695 wild-type isoform (Moechars *et al.*, 1999), providing strong evidence for a direct role of APP metabolites and against amyloid plaques that never formed in these mice. The APP[V717I] transgenic mice studied over the last years and as presented here, are therefore perfect tools to study the early cognitive defects in relation to the early and late biochemical characteristics of the induced AD-like amyloid pathology.

5. MODULATING AMYLOID PEPTIDE PRODUCTION: THERAPEUTIC VALUE?

Presenilin-1 (PS1) is essential in the generation of amyloid peptides from APP (De Strooper *et al.*, 1998) either by exerting the proteolytic activity itself or by acting as an essential component of the complex that constitutes γ -secretase. The crucial and causal role of PS1 in the development of amyloid pathology has also made it a primary therapeutic target, despite the fact that inactivation of the PS1 gene resulted in embryonic death due to pleomorphic effects, *i.e.* disturbed somitogenesis, cranial hemorrhages, impaired neurogenesis, cerebral cavitations and malformation of the brain (Wong *et al.*, 1997; Shen *et al.*, 1997; De Strooper *et al.*, 1998).

To allow us to study the function of PS1 in adult brain, we have generated conditional PS1-knockout mice, with a post-natal, neuron-specific deletion of PS1 not impairing their health, fertility nor normal life-span up to the age of 2 year (Dewachter *et al.*, 2002). Subsequently, these PS1(n^{-/-}) mice were crossed with the APP[V717I] transgenic mice described above to define the effect on the amyloid pathology. In the APP[V717I] x PS1(n^{-/-}) double transgenic mice, the neuronal absence of PS1 effectively prevented the formation of all amyloid pathology. In contrast to the parental APP[V717I] transgenic mice, no thioflavine-S-reactive amyloid plaques nor immuno-reactive diffuse amyloid deposits were detected in the brain of double transgenic mice. The fact that the neuron-specific inactivation of PS1 *in vivo* was positive and had no major adverse effects on these animals proved that adult neurons can manage without PS1, at least in mice and possibly because PS2 took over some functions. Moreover, the prevention of both types of amyloid pathology, *i.e.* amyloid plaques and CAA, again demonstrated their common origin (Dewachter *et al.*, 2002).

The inactivation of PS1 effectively inhibited the formation of amyloid peptides but caused the accumulation of their obligate precursor, *i.e.* the C99 or β -CTF or β -C-terminal fragments of APP. Although it is hardly likely that these have a physiological function on their own, it has been postulated, based on cellular studies, that these CTF can be neurotoxic (for review see Dewachter and Van Leuven, 2002). That could explain our unexpected observations that the inhibition of amyloid peptide formation did actually not improve, but aggravated the cognitive defects even of young APP[V717I] x PS1(n^{-/-}) double transgenic mice, relative to age matched APP[V717I] single transgenic mice (Dewachter *et al.*, 2002). That outcome confirmed the negative expectations from short-term experiments with γ -secretase inhibitors that these were not effective, and moreover very toxic (Wong *et al.*, 2004).

In another vein, a recent collaborative study aimed to define the role of the desintegrin and metalloproteinase ADAM10 as the major α -secretase in the non-amyloidogenic pathway, by cleaving membrane bound APP in the middle of the amyloid region to prevent formation of amyloid peptides. In double transgenic mouse models, we provided the first evidence that ADAM10 effectively acted as an α -secretase in vivo (Postina *et al.*, 2004; see also Chapter 6)). Neuronal co-expression of wild type ADAM10 or of a catalytically inactive dominant-negative mutant together with APP[V717I] in double transgenic mice, respectively stimulated and inhibited the non-amyloidogenic processing of APP. Beneficial results of enhanced α -secretase cleavage of APP were the increased production of secreted neurotrophic APPs α , concomitant with reduced formation of amyloid peptides and an almost complete prevention of their deposition into amyloid plaques at old age. Moreover, the cognitive defects of the parental APP[V747I] transgenic mice were improved by co-expression of ADAM10 (Postina *et al.*, 2004). Inversely, co-expression of the dominant-negative mutant of ADAM10 led to increased amyloid plaques in the brain of double transgenic mice.

6. CONCLUDING REMARKS AND PROSPECTS FOR AD MODELS: A CASE FOR MULTIPLE TRANSGENIC MICE?

The senile plaques and cerebral amyloid angiopathy in the APP[V717I] mouse exhibit a very striking similarity to those observed in familial and sporadic AD patients at old age. The morphological pattern, the ultrastructural aspects and the biochemical composition of senile plaques and vascular amyloid deposits in humans are reproduced in an almost identical manner in the APP[V717I] transgenic mice (Moechars *et al.*, 1999; Van Dorpe *et al.*, 2000; Dewachter *et al.*, 2002). In humans, AD is characterized by a protracted clinical course covering up to 20 years while the lifespan of a laboratory mouse does not exceed more than 2 years. It is therefore surprising that the entire pathological history of the disease can be compressed into about 18 months, but presenting opportunities and advantages for further investigations into the pathogenesis of this devastating neurodegenerative disease. In particular, we anticipate to be able to identify new markers, targets and procedures for the early and objective diagnosis, allowing us to test novel therapeutic drugs and strategies in these pre-clinical models. They will help to accelerate drug discovery, leading to the recognition of therapeutic agents that are effective in postponing the onset or slowing the progression of neurodegenerative disease.

Whereas amyloid pathology in all its aspects in the brain of AD patients has now been approximated or even "copied" almost exactly by overexpression of mutant forms of APP, with or even without PS1, a similar strategy failed to yield neurofibrillary tangles in brain of transgenic mice - thus far. Generating an animal model for Alzheimer's disease that contains or develops all the neuropathological features was and is an enormous challenge. Following up the current amyloid success, models are needed to include the evident progression with age as in patients, with matching topology and regional distribution and including quantitative characteristics that parallel the human disease.

The successful generation of mice with amyloid plaques and angiopathy that actually seem to miss only the neurofibrillary tangles to show the complete AD-pathology, is evidently very encouraging. Moreover, the combination with other transgenic mouse strains, *i.e.* PS1 mutant, PS1(n/-), ADAM10, BACE, APP-/-, ApoE4, have provided us with invaluable data, impossible to otherwise obtain. This evidently makes a strong case for more multiple transgenic mice.

To further explore the relation between the amyloid and tau-pathologies, we have generated APP[V717I] x PS1 x tau-4R triple transgenic mice. Surprisingly, the resulting offspring suffered very high premature mortality, surviving between 6 to maximally 20 weeks; this prevented extensive analysis of the phenotype of adult and aged mice. We confirmed that these mice are extremely sensitive to external environmental stress as reported by others, although the exact cause of death was not evident (Pedersen *et al.*, 1999). Subsequently we have modeled FTD by generating tau-P301L transgenic mice (Terwel *et al.*, unpublished results) and combined these with the APP[V717I] into double transgenic mice. Although these experiments are ongoing, these mice do not appear to develop a really synergistic pathology (Muyllaert *et al.*, unpublished results), as claimed to be observed in similar models (Lewis *et al.*, 2001).

Despite the lack of neurofibrillary tangles in any of our APP single or double transgenic mice, the mice allowed us to study and explore APP-processing *in vivo*, in particular at a young age. The double transgenic APP[V717I] x BACE, APP[V747I] x ADAM10 and APP[V717I] x PS1[A246E] mice are excellent tools to further investigate the origin and repercussions of the amyloid peptides, both at young and old age.

The mechanism(s) by which neurons become non-viable and are eventually "executed" in AD is still in need of detailed understanding at the molecular level. The reported and discussed findings do emphasize the role and importance of clearance pathways of amyloid peptides (Vekrellis *et al.*, 2000; Li *et al.*, 1999) and of the involvement of ApoE-lipoproteins and its receptors in brain (Tesseur *et al.*, 2000a,b; May and Herz, 2003). It is clear

that our continued effort to model all aspects of the pathology of AD will eventually have to answer these and more fundamental questions that are bound to surface. We believe that a continued effort in transgenesis and in combined mouse models will further prove of great value to understand the interactions of human genes, or rather proteins *in vivo*, that can only be studied in multiple transgenic mice.

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