



Occurrence and co-localization of amyloid β -protein and apolipoprotein E in perivascular drainage channels of wild-type and APP-transgenic mice

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Abstract

The deposition of the amyloid β -protein (A β) is a hallmark of Alzheimer's disease (AD). One reason for A β -accumulation and deposition in the brain may be an altered drainage along perivascular channels. Extracellular fluid is drained from the brain towards the cervical lymph nodes via perivascular channels. The perivascular space around cerebral arteries is the morphological correlative of these drainage channels. Here, we show that A β is immunohistochemically detectable within the perivascular space of 25 months old wild-type and amyloid precursor protein (APP)-transgenic mice harboring the Swedish double mutation driven by a neuron specific promoter. Only small amounts of A β can be detected immunohistochemically in the perivascular space of wild-type mice. Cerebrovascular and parenchymal A β -deposits were absent. In APP-transgenic mice, large amounts of A β were found in the perivascular drainage channels accompanied with cerebrovascular and parenchymal A β -deposition. The apolipoprotein E (apoE) immunostaining within the perivascular channels did not vary between wild-type and APP-transgenic mice. Almost 100% of the area that represents the perivascular space was stained with an antibody directed against apoE. Here, A β co-localized with apoE indicating an involvement of apoE in the perivascular clearance of A β . Fibrillar congophilic amyloid was not seen in wild-type mice. In APP-transgenic animals, congophilic fibrillar amyloid material was seen in the wall of cerebral blood vessels but not in the perivascular space. In conclusion, our results suggest that non-fibrillar forms of A β are drained along perivascular channels and that apoE is presumably involved in this clearance mechanism. Overloading such a clearance mechanism in APP-transgenic mice appears to result in insufficient A β -clearance, increased A β -levels in the brain and the perivascular drainage channels, and finally in A β -deposition. In so doing, our results strengthen the hypothesis that an alteration of perivascular drainage supports A β -deposition and the development of AD. © 2006 Elsevier Inc. All rights reserved.

Keywords: Alzheimer's disease; Amyloid β -protein; Apolipoprotein E; Perivascular drainage

1. Introduction

Alzheimer's disease (AD) is histopathologically characterized by the deposition of amyloid β -protein (A β) [4,16].

Increased levels of A β are considered to be responsible for neurodegeneration in AD [9]. An increase of A β in the brain can either result from increased production or from decreased clearance of A β [15]. Transgenic mice overexpressing mutant amyloid precursor protein (APP) produce increased levels of A β and develop A β -plaques and cerebral amyloid angiopathy (CAA) [7,10,29]. APP23 mice overexpress human APP

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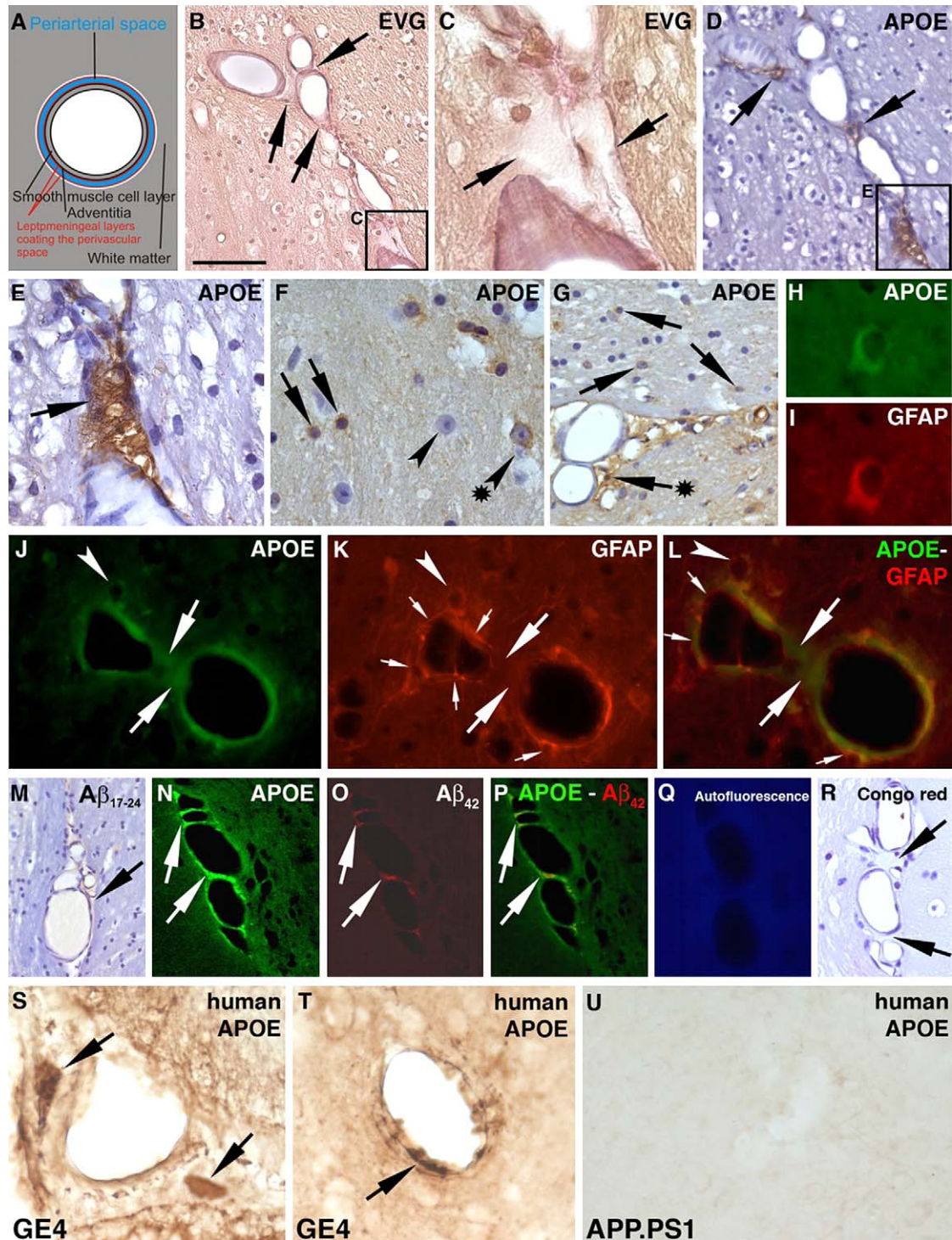


Fig. 1. ApoE and A β within the perivascular drainage channels in wild-type and GE4 mice. (A) Schematic representation of a white matter artery in the basal ganglia-thalamus region. The perivascular drainage channel (blue) is coated by two leptomeningeal layers (red). The inner layer borders the adventitia, the outer layer adjoins the extracellular space of the brain, e.g. the white matter [24]. (B) The elastica van Gieson staining (EVG) shows the posterior cerebral artery and its ramifications. Between these vessels the perivascular drainage channels are visible in the 25 months old wild-type mouse (arrows). (C) At higher magnification proteinaceous material is seen within these channels (arrows). (D) The perivascular spaces are almost entirely filled with anti-apoE-positive material (arrows). (E) At the higher magnification level, anti-apoE-positive material co-localizes the proteinaceous material within the perivascular space (arrow) as documented in the adjacent EVG and anti-apoE-stained sections (B–E). (F) Glial cells (arrows) and a single neuron (arrowhead with star) show apoE positivity whereas another neuron (arrowhead) does not exhibit apoE. Neurons are identified by the nucleus containing a prominent nucleolus as well as by the presence of neuritic stumps. (G) ApoE-positive glial cells are located within the neuropil (arrows) and in association with the perivascular space (arrow with star). (H and I) Double label immunofluorescence indicates the astrocytic nature of the apoE containing glial cells by the colocalization of apoE (H) and GFAP (I). (J–L) Perivascular

harboring the Swedish double mutation (670/671 KM →NL) driven by the neuron specific Thy-1 promoter [29]. APP-overexpression is not seen in other tissues except the central nervous tissue of these mice [29]. Therefore, the APP23 mouse is an ideal model for studying the mechanisms of A β -deposition and A β -clearance. One clearance mechanism for A β from brain is binding to apolipoprotein E (apoE) [28] and the subsequent uptake by astrocytes [13]. However, apoE is also found in senile plaques in humans [20] and mice [22] and appears to be involved in the formation of newly formed plaques [31]. In addition to the cellular clearance of A β by astrocytes and microglial cells [11,13] and the enzymatic degradation by neprilysin and/or insulin degrading enzyme [11,19], drainage of extracellular A β along perivascular spaces has been discussed to play a significant role in A β -clearance [36]. Perivascular channels represent drainage channels for extracellular fluid from the brain towards the cervical lymph nodes [35,40]. The perivascular space around cerebral arteries is the morphological correlative of these drainage channels [35,40] (Fig. 1A). Although the development of CAA in transgenic mice overexpressing APP through a neuron-specific promoter strongly suggests clearance of A β along the perivascular channels [3], A β has not been shown to occur physiologically in these channels and it is not clear which forms of A β are drained.

Therefore, the aim of this study is to address the question whether non-fibrillar A β is present within the perivascular space of cerebral vessels and to examine the role of apoE in the perivascular drainage of A β .

2. Material and methods

To demonstrate the presence of A β within the perivascular space and to test whether apoE is involved in this drainage process, we studied brains from 25 months old, female wild-type ($n=20$) and APP23 mice ($n=16$) for the presence of A β and apoE within the perivascular channels of cerebral vessels. Animals were treated in agreement with the German law on the use of laboratory animals. Perfusion fixation of the animals was performed transcardially with Tris buffered saline (TBS) with heparin (pH 7.4) followed by the injection of 0.1 M phosphate buffered saline (PBS) (pH 7.4) containing 2.6% paraformaldehyde. Subsequently, brains were removed and fixed in a 2.6% paraformaldehyde solution for 2 weeks. Sections from the hemispheres, the brain stem and

the cerebellum were embedded in paraffin and microtomed at 4 μ m thickness. Paraffin sections were stained with Hematoxylin and Eosin, Congo red, and the Elastica van Gieson method.

Immunohistochemistry for the detection of A β was performed with an antibody raised against A β_{17-24} detecting both human and mouse A β (Signet (Dedham, MA, USA), 4G8, 1/5000, formic acid pretreatment [12]). ApoE was recognized with a polyclonal antibody directed against mouse apoE (Santa Cruz (Santa Cruz, CA, USA)), polyclonal goat, 1/100, microwave and formic acid pretreatment [39]). Primary antibodies were detected with biotinylated secondary antibodies, ABC-complex, and 3,3 diaminobenzidine-HCl (DAB-HCl, Sigma-Aldrich (Taufkirchen, Germany)). To block intrinsic mouse IgG when working with primary antibodies raised in mouse sections were treated with unlabeled anti-mouse IgG (Biomedica (Foster City, CA, USA), polyclonal goat, 1/100) prior to the incubation with the primary antibody. Counterstaining with hematoxylin was performed afterwards. Sections were mounted in Corbit-Balsam (Hecht, Hamburg, Germany). Positive, negative, and blank controls were performed. To validate blocking for intrinsic mouse IgG when using antibodies produced in mouse we treated sections from APP23 mice with anti-A β_{17-24} . These sections were, then, incubated with an unlabeled goat anti-mouse IgG antibody for blocking immunoreactivity followed by detection of mouse IgG with a biotin-labeled anti-mouse IgG secondary antibody, ABC-complex, and DAB-HCl. Blocking was successful when these sections including the amyloid plaques remained unstained while the positive control was working (online attachment Fig. O-1). To confirm the presence of apoE co-localizing A β and to identify the role of astrocytes for perivascular apoE, double label immunohistochemistry was performed in wild-type and APP23 mice using the polyclonal anti-apoE antibody combined with (1) anti-A β_{17-24} , (2) an antibody directed against A β_{42} (MBC42, 1/200, formic acid pretreatment [38]), (3) an antibody against A β_{40} (MBC40, 1/20, formic acid pretreatment [38]), and (4) a monoclonal antibody directed against the glial fibrillary acidic protein (GFAP, Boehringer-Mannheim (Mannheim, Germany), G-A-5, 1/20). Additional double staining was done to detect A β and astrocytic processes simultaneously with anti-A β_{42} and anti-GFAP (DAKO (Glostrup, Denmark), polyclonal-rabbit, 1/1000). Intrinsic mouse IgG was blocked with unlabeled anti-mouse IgG. Primary antibodies were visualized with Carbocyanin 3 (Cy3)-labeled antibody.

Fig. 1. apoE-positive cells also co-localize GFAP (arrowheads). Astrocytic foot processes frequently co-localize apoE bordering the adventitia of the vessels (small arrows). However, the perivascular space is negative for GFAP but exhibits apoE-positive material (large arrows). (M) In wild-type animals, only small amounts of A β are detectable with an antibody raised against A β_{17-24} in the perivascular space of wild-type animals (arrow). (N–Q) Double label immunofluorescence with antibodies raised against apoE (N) and A β_{42} (O) confirms the presence of apoE and small amounts of A β within the perivascular space of wild-type mice. A β co-localized apoE within the perivascular channels (arrows) (P). Excitation with UV-light does not induce fluorescence of the material positive for apoE and A β_{42} , thereby excluding that the staining results are due to unspecific autofluorescence (Q). (R) The negative staining for Congo red indicates the absence of fibrillar amyloid in the perivascular channels of wild-type animals (arrows). (S and T) In GE4 mice expressing human apoE 4 driven by an astrocyte-specific GFAP promoter human apoE is detected in the perivascular spaces of larger (S) and smaller vessels (T) (arrows). (U) The antibody used for the detection of human apoE does not stain significant amounts of mouse apoE as shown in an APP.PS1 double transgenic mouse not expressing human apoE. Calibration bar: (B, D, G) 70 μ m; (C, E, F) 40 μ m; (H–L) 15 μ m; (M): 80 μ m; (N–R) 45 μ m; (S) 35 μ m; (T), (U) 25 μ m.

ies directed against goat IgG (prior to the use of anti-mouse IgG from goat), Carbocyanin 2 (Cy2) or Cy3-labeled antibodies against mouse IgG, and Cy2-labeled antibodies directed against rabbit IgG (Cy2 and Cy3, Dianova (Hamburg, Germany), 1/50). These sections were mounted in Corbit-Balsam without counterstaining. To confirm specific detection of A β with the monoclonal antibodies raised in wild-type and APP23 mice additional sections were also incubated with a polyclonal antibody raised against the peptide A β _{1–42} (polyclonal rabbit, 1/750, formic acid pretreatment [37]). Sections from 18 to 26 months old APP-knockout mice [3] were stained with all anti-A β antibodies and proved the absence of unspecific labeling (online attachment Fig. O-2). Additionally, blank controls for unspecific binding with monoclonal mouse antibodies were performed with an antibody directed against smooth muscle cell actin (Dianova, 1A4, 1/100).

To study whether brain-derived apoE can be potentially involved in clearance mechanisms of the brain we investigated four 15 months old female transgenic mice, which express human apoE 4 through a GFAP-promoter (GE4-mice [34]). These animals were treated in agreement with the Belgium law on the use of laboratory animals. Vibratome sections of the brains from perfusion fixed animals were used. ApoE was detected in these mice with an antibody that specifically labels human but not mouse apoE (Signet, D6E10, 1/500, microwave and formic acid pretreatment) after blocking intrinsic mouse IgG. The primary antibody was detected with a biotinylated secondary antibody against mouse IgG and the ABC-detector complex as described above. A counterstaining was not performed. Sections were mounted in Corbit-Balsam. As negative control vibratome sections from APP[V717I]-PS1[A246E]-transgenic mice (APP.PS1; [34]) were stained for human apoE simultaneously.

To confirm and to demonstrate the presence of A β within the perivascular space by immuno electron microscopy three female APP23 mice at 15 months of age were perfusion fixed as described. To allow sensitive antibody binding vibratome sections of 100 μ m thickness were flat embedded in LR-White (Polyscience, Inc., Warrington, PA, USA) without osmium-fixation. Samples of the frontocentral cortex and the adjacent white matter were pasted on Epon blocks and semi-thin and ultra-thin sections were cut. Semi-thin sections were stained with methylene blue and mounted in Corbit-Balsam. Ultra-thin sections were absorbed to nickel grids. The grids were dried and immunostained with anti-A β _{1–17} (Signet, 6E10, 1/100) after blocking for mouse IgG. The primary antibody was detected with anti-mouse IgG conjugated with gold particles. Block staining with uranyl acetate and lead citrate has not been performed. The sections were viewed with a Philips CM10 transmission electron microscope (Eindhoven, Netherlands). Pictures were taken with a SIS digital imaging system (Münster, Germany).

To estimate the amount of apoE and A β within the perivascular space we semiquantitatively assessed whether and to

which degree the perivascular space was stained with anti-apoE and anti-A β _{17–24}. The following criteria were used for the semiquantitative assessment of the perivascular apoE- and A β -load in immunostained sections: 0 = no perivascular immunopositive material; 1 = less than 50 % of the perivascular space was stained with a given antibody, 2 = 50% and more of the perivascular space was stained with a given antibody. The Mann–Whitney *U*-test was used to compare differences in the perivascular apoE- and A β -load between wild-type and APP23 mice. Differences between the perivascular apoE- and A β -load were tested separately for wild-type and APP23 mice using the Sign-test. Statistical tests were calculated with the SPSS 11.0 program (SPSS, Chicago, IL, USA). *p*-Values were corrected for multiple testing when appropriate.

3. Results

Microscopic analysis of cerebral vessels revealed visible perivascular channels in all animals (Fig. 1B and C). The perivascular space was best seen at the level of the hippocampal formation around the posterior cerebral artery and its ramifications. Near and within perivascular channels no cellular reaction was detected (Fig. 1B and C). Only amorphous proteinaceous material was visible in the Hematoxylin and Eosin and Elastica van Gieson stained sections (Fig. 1C). Nearby blood vessels did not contain proteinaceous material in the lumen because the blood was washed out due to perfusion fixation.

Wild-type mice exhibited anti-apoE-positive material within the perivascular space (Fig. 1D, E, J, L). ApoE was detected in those areas of the perivascular space which exhibited proteinaceous material in adjacent hematoxylin and eosin or elastica van Gieson stained sections (Fig. 1C and E). The perivascular channels were almost entirely stained with apoE-immunoreactive material (Fig. 1D and E). In addition to perivascular space-apoE, astrocytes of the white matter as well as a few neurons exhibited apoE (Fig. 1F–I). Neurons were identified by their characteristic morphology with proximal stumps of dendrites and a prominent nucleolus. ApoE-positive and apoE-negative neurons were located next to one another (Fig. 1F). Double label immunohistochemistry confirmed the presence of apoE positive astrocytes by the cellular co-localization of apoE and GFAP (Fig. 1H, I–L). Some apoE-positive astrocytes bordered the perivascular space (Fig. 1G, J, K, L). In addition, perivascular astrocytic foot processes were identified by the expression of GFAP and contained apoE-positive material (Fig. 1J–L, 2). In sections stained with anti-A β _{17–24} (Fig. 1M), anti-A β ₄₂ (Fig. 1O and P), anti-A β ₄₀, and anti-A β _{1–42}, only a very weak staining in less than 50% of the perivascular space positive for apoE was observed (Fig. 1N–P). Within the perivascular space A β co-localized with apoE. Excitation with ultraviolet light did neither show unspecific autofluorescence nor amyloid material within the perivascular channels (Fig. 1Q). The Congo red

staining for the detection of fibrillar amyloid material [8] was negative (Fig. 1R). A β -plaques and CAA were not seen in wild-type animals. APP-knockout mice did not show a staining of the perivascular space when incubated with all anti-A β antibodies (online attachment Fig. O-1). Sections incubated with monoclonal antibodies directed against smooth muscle cell actin and GFAP did not show an unspecific binding of the antibody in the perivascular space.

GE4 mice expressing human apoE 4 through a GFAP-promoter only in the central nervous system exhibited significant amounts of apoE within the perivascular space detectable with the D6E10 antibody specific for human apoE (Fig. 1S and T) whereas control sections from APP.PS1 mice not exhibiting human apoE showed only a negligible coloration (Fig. 1U).

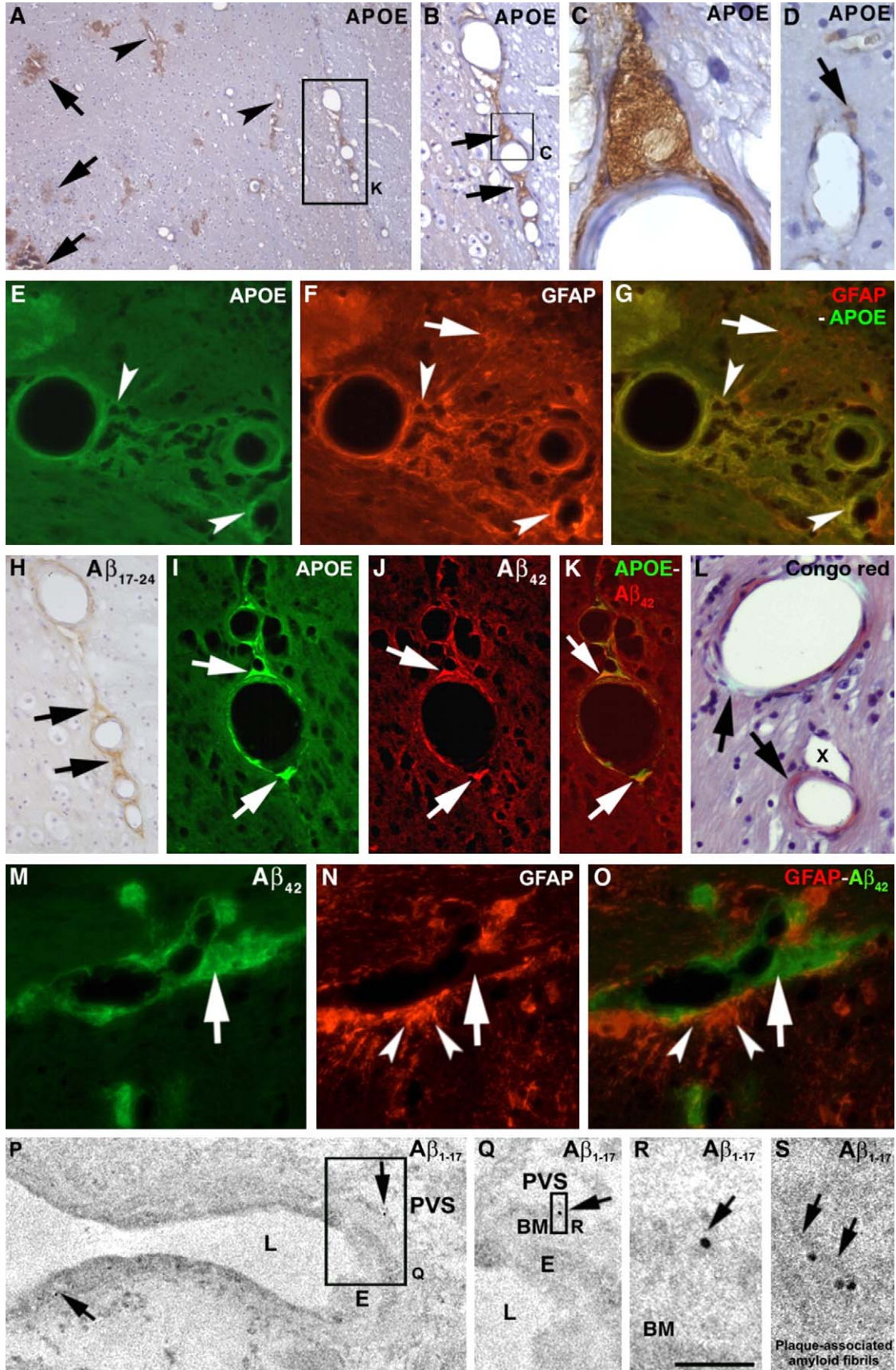
In APP23 mice, apoE was found within perivascular channels as well as in amyloid plaques and CAA (Fig. 2A–C). The perivascular channels were almost entirely stained with anti-apoE-positive material (Fig. 2C). Moreover, apoE-positive glial cells were found in association with the perivascular space of cerebral vessels (Fig. 2D). Double labeling for apoE and GFAP confirmed this association. Astrocytic foot processes surrounding the blood vessels also frequently exhibited apoE (Fig. 2E–G). Anti-A β _{17–24}, anti-A β ₄₂, and anti-A β ₄₀ detected A β within the perivascular space of APP23 mice, stained more than 50% of this compartment (Fig. 2H–K), and co-localized with apoE in double stained sections (Fig. 2K). Vascular A β -deposits bordering the perivascular space exhibited Congo red positive fibrillar amyloid showing the characteristic birefringence under polarized light (Fig. 2L). The perivascular space itself did not exhibit Congo red-positive material. There was no colocalization of A β and GFAP within the perivascular space (Fig. 2M–O). Senile plaques and CAA-affected vessels were detected with all anti-A β antibodies used in this study as well as with anti-apoE as documented by double label immunofluorescence.

Immunoelectron microscopy confirmed the presence of A β _{1–17}-positive material within the perivascular space (Fig. 2P–R). A β _{1–17}-positivity was associated with proteinaceous material (Fig. 2Q and R). This material was shown in 15 months old mice in the perivascular space of vessels without vascular amyloid deposits, i.e. in the absence of amyloid fibrils positive for anti-A β _{1–17}, and did not exhibit the morphology of amyloid fibrils (Fig. 2R and S).

Semiquantitative analysis of the perivascular apoE- and A β -load revealed that there were no significant differences in the apoE-load between wild-type and APP23 mice (Mann–Whitney *U*-test: $p=0.367$) (Fig. 3). On the other hand, APP23 mice showed a higher perivascular A β -load than wild-type animals (Mann–Whitney *U*-test: $p<0.0001$). Comparing perivascular apoE- and A β -load, there were no differences in APP23 mice (Sign-test: APP23 $p=0.625$). In the wild-type mice, the perivascular A β -load was lower than the perivascular apoE-load (Sign-test: $p<0.01$) (Fig. 3).

4. Discussion

The presence of A β within the perivascular space of wild-type and APP23 mice strongly supports the hypothesis of Weller et al. [36] that drainage along these channels contributes to the clearance of A β from brain. Physiologically, low amounts of A β were detected immunohistochemically within these channels in wild-type mice. The absence of Congo red stained material indicates the non-fibrillar nature of the A β -positive material in these mice. As soon as the amount of neuronal derived A β is increased in the brains of 25 months old APP23 mice [29] more than 50% of the perivascular channels were stained with different antibodies detecting A β . Fibrillar amyloid material was identified within the bordering vessel walls using the Congo red staining method. However, A β within the perivascular space did not appear to be fibrillar amyloid as demonstrated by the absence of congophilic material within the perivascular channels in 25 months old mice and by the detection of anti-A β immunoreactive proteinaceous, non-fibrillar material in the perivascular space in 15 months old APP23 mice at the electron microscopic level. Therefore, it is tempting to speculate that the perivascular drainage of non-fibrillar, presumably soluble forms of A β is overloaded when APP23 mice develop A β -deposits in the brain and along cerebral blood vessels. A second argument in favor of this hypothesis is that fibrillar amyloid deposits in the vessel wall of APP23 mice result from increased neuronal A β -production [3] and the only way for A β to be deposited at the wall of leptomeningeal vessels in these mice is its drainage along perivascular channels. Wild-type mice never developed vascular amyloid-deposits at this age. However, production of A β in vascular smooth muscle cells [5,6] may contribute to the occurrence of perivascular non-fibrillar A β in wild-type mice. Here, apoE–A β complexes can accumulate and form deposits within smooth muscle cells [18]. Such deposits can arise from endogenous A β -production in smooth muscle cells but also from exogenous A β [17]. Thus, the increase in perivascular A β -load seen in APP23 mice is apparently due to non-smooth muscle cell derived, neuronal A β -production because the transgene in APP23 mice is not expressed in other tissues than nervous tissue [29]. Nevertheless, smooth muscle cells may be involved in the formation of apoE–A β complexes when exogenous A β occurs in the perivascular space as seen in tissue culture experiments [17]. An alternative explanation is that apoE produced by astrocytes [2] is secreted by astroglial perivascular foot processes, drained along the perivascular space and interacts with A β . Our finding that human apoE occurs in the perivascular space of GE4 mice expressing human apoE only in the brain driven by an astrocyte-specific GFAP-promoter indicates that astrocyte-derived apoE is indeed drained along perivascular channels. In wild-type as well as in APP23 mice apoE positive astrocytes were seen. Some astrocytes with foot processes bordering the perivascular space exhibited apoE-positive foot processes. These finding together with the presence of human apoE in the perivascular channels of GE4



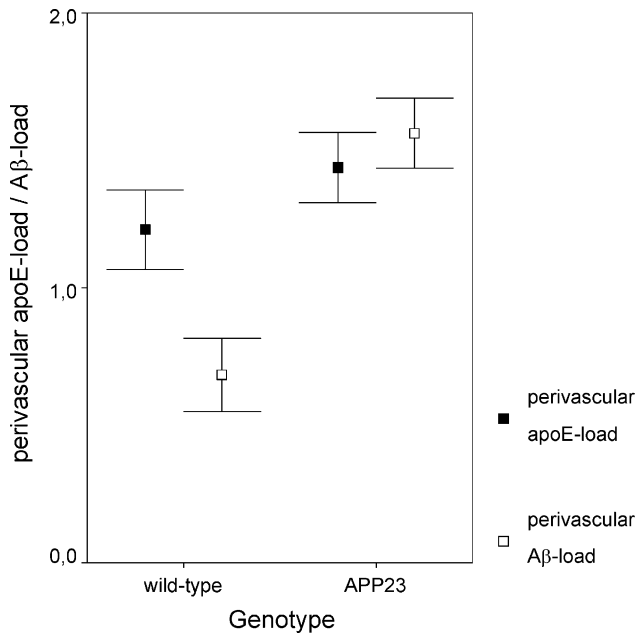


Fig. 3. Perivascular apoE-load and A β -load in wild-type and APP23 mice. In wild-type mice the perivascular apoE-load is higher than the perivascular A β -load (Sign-test: $p < 0.01$). In APP23 mice the perivascular A β -load increases (Mann–Whitney U -test: $p < 0.0001$) and reaches the level of the perivascular apoE load (Sign-test: APP23 $p = 0.625$). The perivascular apoE-load does not increase significantly in APP23 mice (Mann–Whitney U -test: $p = 0.367$).

mice point to the involvement of astrocytes in the production and drainage of apoE into perivascular channels. However, although our results provide evidence for the contribution of astrocyte-derived apoE and neuron-derived A β in perivascular drainage further studies are required to address the specific contribution of different cell types and proteins in this process.

The specificity of the immunohistochemical results was confirmed: unspecific binding of the monoclonal mouse antibodies has been excluded by negative staining results in APP-knockout mice, the absence of an unspecific binding with a monoclonal mouse-antibody against smooth muscle cell actin, and by the detection of A β within the perivascular spaces with a polyclonal rabbit-antibody directed raised against A β_{1-42} . The antibody used for the detection of human apoE specifically labeled human apoE but not mouse apoE.

The co-localization of A β and apoE within the perivascular channels presumably indicates an involvement of apoE in the perivascular drainage of A β . Since apoE binds A β [28] it appears likely that A β undergoes drainage along perivascular channels in form of apoE–A β complexes. Such complexes were found in the brains of AD patients as well as in those of non-demented individuals [21,25]. In wild-type mice a relatively higher apoE load is detected within the perivascular space compared with the perivascular A β -load. Thus, clearance of presumable apoE–A β -complexes does not appear to be saturated in these animals. Overloading of the A β -drainage capacity of the perivascular channels appears to result in the deposition of A β in APP-transgenic mice as shown here. The hypothesis that apoE–A β -complexes are involved in perivascular A β -drainage and deposition is supported by our finding of a co-localization of apoE and A β within the perivascular channels. The occurrence of apoE in the plaques seen in the APP23 mice supports the hypothesis that apoE–A β complexes not drained via periarterial channels are apparently involved in the generation of A β -plaques. Further arguments favoring this hypothesis are that apoE co-localized with A β in newly formed plaques in humans suggesting an involvement of apoE–A β complexes in the initiation of A β -deposition [31] and that the severity and type of A β -deposition in blood vessels depends on the apolipoprotein

Fig. 2. ApoE and A β within perivascular drainage channels in APP23 mice. (A) The thalamic region of an APP23 transgenic mouse exhibits anti-apoE positive amyloid plaques (arrows), CAA with anti-apoE positive amyloid deposits in the vessel wall (arrowheads) and apoE within the perivascular space of the posterior cerebral artery and its branches (boxed area). (B) The enlargement of the boxed area of the anti-apoE stained section from the thalamic region of an APP23 mouse shows perivascular drainage channels almost entirely filled with anti-apoE-positive material (arrows). (C) High power analysis indicates that the apoE-positive proteinaceous material within the perivascular does not morphologically differ from that found in wild-type mice (Fig. 1E). (D–G) Similar to wild-type mice apoE-positive astrocytes are seen. Some are located in the vicinity of the perivascular space (arrow in D). Other astrocytes not directly located near the perivascular space send apoE-positive foot processes to the blood vessels (arrowheads in E–G) although the cell body of the astrocytes does not exhibit apoE-positivity (arrow in F and G). (H) In contrast to wild-type mice, the perivascular space of APP23 mice is also almost entirely filled with A β as detected with an antibody raised against A β_{17-24} (arrows) as seen in the consecutive section to B. (I–K) Double label immunofluorescence with antibodies against apoE (I, K), and A β_{42} (J, K) confirms that A β within the perivascular channels co-localizes with apoE. Since anti-A β_{42} does not cross-react with APP [30] these staining results prove the presence of the A β -peptide within the perivascular space. In the APP23 mouse more than 50% of the perivascular space is positive for A β and anti-A β_{42} -positive material co-localizing apoE (arrows in I–K). (L) Fibrillar amyloid was detected with the Congo red method in vessel walls of APP23 mice bordering the perivascular space. Note the red-green birefringence of the Congo red positive amyloid (arrows). The perivascular space itself did not contain congophilic material (X). (M–O) Perivascular A β positive for anti-A β_{42} (arrow) does not co-localize with GFAP-positive astrocytes or astrocytic foot processes bordering the perivascular space (arrowheads). (P–R) Ultrastructural analysis of anti-A β_{1-17} -positive material within the perivascular space. LR-white embedded ultra-thin sections were immunolabeled with gold particles and analyzed without further contrast. (P) Anti-A β_{1-17} -positive material (arrows) is found in the perivascular space (PVS) near the basement membrane but not in endothelial cells (E) or in the vessel lumen (L) of a perfusion-fixed 15 months old APP23 mouse. The boxed area indicated the area enlarged in Q. (Q) The A β_{1-17} -positive material is found directly beside the basement membrane (BM; arrow). (R) The enlargement of the boxed area in Q shows that the gold-particles labeling A β are associated with proteinaceous non-fibrillar material (arrow). (S) The positive control, i.e. an amyloid plaque in a 15 months old APP23 mouse, indicated the fibrillar structure of the amyloid within the senile plaque (arrows) in comparison to the non-fibrillar appearance of the proteinaceous material in the perivascular space of a small vessel in the APP23 mouse (arrow in R). Calibration bar: (A) 170 μ m; (B) 80 μ m; (C) 15 μ m; (D, M–O): 30 μ m; (E–G) 50 μ m; (H–K) 70 μ m; (L) 40 μ m; (P) 1 μ m; (Q) 666 nm; (R, S) 200 nm.

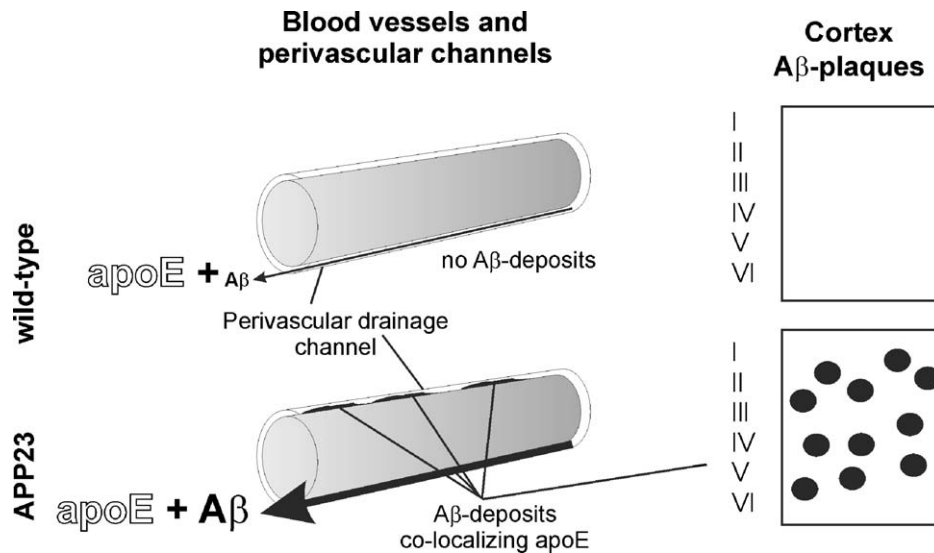


Fig. 4. A β drainage along perivascular channels and its possible relation to A β -deposition in the brain. The perivascular channels drain extracellular fluid of the brain into the cervical lymph nodes [40]. Large amounts of apoE are present within the perivascular drainage channels under physiological conditions in aged wild-type animals. As long as only small amounts of A β are cleared along these channels no amyloid deposition takes place (thin A β arrow). APP23 mice produce large amounts of A β presumably drained along the perivascular channels (thick A β arrow). As soon as the drainage capacity is overloaded cerebrovascular and parenchymal amyloid deposits may appear.

tein E (APOE)-genotype [17,23,27,32]. Such an initial deposition of A β together with apoE may indicate that apoE-A β complexes represent a seeding agent for plaque generation as soon as they cannot be drained sufficiently. Arguments in support of the assumption that apoE-A β -complexes are involved in the initiation of A β -plaque generation are: (1) the reduced number of A β -plaques seen in PDAPP^{+/+}; apoE^{-/-} mice [1] and the reduction of the A β -plaque load by blocking the A β -binding site of apoE [26], (2) newly formed plaques consisting of A β co-localizing with apoE such as the fleecy amyloid are often associated with A β -containing astrocytes indicating the transgression of the astrocytic clearance capacity for apoE-A β complexes before the generation of A β -plaques starts [31,33], and (3) soluble apoE-A β -complexes are less stable in AD patients when compared with non-demented individuals [25]. In so doing, a physiological role of apoE in the clearance of A β does not contradict the role of apoE-A β complexes in the initiation of A β -deposition. However, since PDAPP^{+/+}; apoE^{-/-} mice are capable of developing A β -plaques [1] and apoE negative newly formed plaques can be seen in the human brain [14,31] the apoE-related clearance and deposition of A β is one important pathogenetic event in the plaque formation but alternative pathways for the clearance of A β from brain and for the development of A β -plaques exist.

In conclusion, in this study we show that A β is presumably drained along perivascular channels and that apoE appears to be involved in this clearance mechanism. Overloading this clearance mechanism in aged APP23 mice appears to result in insufficient A β -clearance increased A β -levels in the brain, and finally A β -deposition (Fig. 4). ApoE-linked forms of

A β , thereby, appear to be involved in the initiation of A β -deposition. Since the APOE ϵ 4-allele is the major genetic risk factor for sporadic AD our results support the hypothesis that an alteration of the apoE-associated clearance of A β along perivascular channels may contribute to the pathogenesis of sporadic AD.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2006.05.029.

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