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Blood-brain barrier in Alzheimer dementia and in non-demented elderly

An immunocytochemical study*

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Summary. Peroxidase-antiperoxidase staining of formalin-fixed brain was employed to compare the blood-brain barrier (BBB) function in five patients with Alzheimer's disease/senile dementia of the Alzheimer type (AD/SDAT) and three patients with AD/SDAT combined with multi-infarct dementia (MID/SDAT) with that of six non-demented aged controls. The diffusion of serum proteins through the BBB was visualized with antisera to albumin, prealbumin, immunoglobulin, C1q, C3c and to fibrinogen. A similar patterns of diffusion was seen in AD/SDAT and non-demented aged individuals. Neuron and glial cells were stained with different antisera in the vicinity of the diffusion. Senile (neuritic) plaques were occasionally visualized with antisera to IgG, C1q and C3c but not with antisera to albumin, prealbumin and fibrinogen in both demented and non-demented aged individuals. Neurofibrillary tangles were not labelled with any of the antisera studied. These results indicate that the BBB is compromised equally in AD/SDAT and in the non-demented elderly.

Key words: Blood-brain barrier – Senile plaques – Alzheimer's disease – Non-demented elderly – Immunochemistry

Alzheimer's disease/senile dementia of Alzheimer type (AD/SDAT) is the most common type of dementia which, in autopsy material, accounts for approximately 50%–70% of all the dementia cases [35]. Multi-infarct demintia (MID) and the combined dementia (MID/SDAT) are seen in about 10%–30% of dementia cases [35]. Histophatologically the most common lesions in AD/SDAT cases are neurofibrillary tangles and senile (neuritic) plaques [8, 9, 34, 37]. Tangles and plaques are not exclusively found in AD/SDAT, but are also found, although to a considerably lesser extent in aged individuals without intellectual impairment [35, 36].

The etiology and pathogenesis of AD/SDAT are not understood. The causative factor of this degenerative disease is believed to be of viral, environmental or immunological origin which might gain access to the brain via an altered blood-brain barrier (BBB). Senile plaques are associated with capillaries [23] and contain amyloid of unknown origin [12, 14, 22, 24, 28, 31, 38, 42, 43]. By immunocytochemical techniques different serum proteins such as albumin [29], prealbumin [32], fibrinogen [29], immunoglubulins [13, 17, 29] and different complement factors [13] have been detected in plaque amyloid. Furthermore, in sera of aged humans [11, 26, 40] and animals [25], the presence of anti-brain antibodies has been shown. In the present study using immunocytochemical techniques we demonstrate that the BBB function is not only compromised in AD/SDAT and in MID/SDAT, but also in non-demented elderly.

Materials and methods

Clinical classification

Eight demented individuals age between 65–93 years (mean age 80.7 ± 7.5) were included in this study. The clinical diagnosis was obtained by appropriate clinical examination and based on the DSM-III classification [4]. These patients had been followed for several years, and all of them met the criteria for AD/SDAT. In addition six patients between 67 and 87 years (mean age 77.8 ± 6.9) without any clinical signs of dementia were included as a control group (Table 1).

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Table 1. Description of the cases. Clinical classification

Case no.	Histo-pathological diagnosis	Age/sex	Drugs[a]	Associated diseases cause of death[b]	Hours between death and autopsy	Brain weight grams	Arterio sclerosis[c]	Malacias[d]
Non-demented								
1		67/M	M, N	1, 2, 4, A	50	1340	++	>1
2		72/M	M	2, 4, 8, A	38	1400	–	0
3		77/F	–	4, 8, A	25	1370	++	0
4		79/F	–	4, A	50	1220	+	0
5		85/M	–	3, 5, 9, S	26	1230	+	0
6		87/M	–	4, A	8	1310	+++	>1
Mean ± SD, $n = 6$		77.8 ± 6.9			33 ± 15	1316 ± 63		
Demented								
7	AD	65/M	M, N	3, 5, S	25	1300	+	0
8	SDAT	76/M	T	5, 7, S	43	1400	+	1
9	SDAT	82/M	–	10, 5, 9, S	33	1380	+	0
10	SDAT	84/M	M, N	5, 7, S	52	1400	+	0
11	SDAT	93/M		3, 5, 7, S	8	1210	+	0
12	MID/SDAT	79/M	M, N	3, 5, S	6	1460	++	1
13	MID/SDAT	82/M	–	3, 5, 6, S	19	1360	+	>1
14	MID/SDAT	85/M	M, N	4, 5, 9, S	19	1240	++	0
Mean ± SD, $n = 8$		80.7 ± 7.5			25 ± 15	1335 ± 72		

AD: Alzheimer's disease; SDAT: senile dementia of the Alzheimer type; MID: multi-infarct dementia

[a] M: Morphine, N: neuroleptics; T: TBC-statics

[b] 1: Hypertonia; 2: angina pectoris; 3: incompensatio cordis; 4: infarctus myocardii; 5: circulatory failure; 6: carcinoma; 7: bronchopneumonia; 8: oedema pulm; 9: infarctus pulm; 10: hypotonia. A: Acute; S: slow way of death

[c] Degree of arteriosclerosis of basal vessels of the circle of Willis: – non, + moderate, ++ severe without lumen obliteration, +++ severe with lumen obliteration

[d] Number of malacias: 0, 1, >1

Neuropathology

After removal of the brain at autopsy, the brains were weighed and gross examined. The degree of arteriosclerosis as well as encephalomalacias and other abnormalities were noted (Table 1).

Histopathology

For the present study tissue was taken from the hippocampus and the frontal lobe, fixed in 10% neutral buffered formalin and embedded in paraffin blocks. The fixation time ranged from 2 weeks to 2 months with an average of 1 month. Six-micrometer sections were mounted on albumin-coated slides and stained for histopathology or immunocytochemistry (see below).

The density of plaques and tangles was evaluated from Bodian-PAS silver-stained [7] sections; microscopical infarctions were examined in haematoxylin-eosin-stained sections. The histopathological criteria for AD/SDAT in this study were the presence of many plaques and tangles in the hippocampus and in the frontal cortex. Five patients fulfilled these criteria. Three of the patients, in addition, showed widespread microscopical infarctions in the frontal cortex and in the hippocampus and thereby met the criteria for both MID and SDAT and formed the separate group of combined dementia (MID/SDAT).

Immunocytochemistry

Deparaffinized and rehydrated sections were washed in 0.05 M sodium phosphate-buffered saline, pH 7.2 (PBS) for 45 min (three changes, 4 l each) and examined for the presence of serum proteins using the peroxidase-antiperoxidase (PAP) technique [33] with the following modifications. After the preincubation with 3% normal goat serum (Nordic, Sweden) in PBS containing 2% bovine serum albumin (BSA) for 15 min at room temperature, the sections were incubated with primary antisera overnight at 5° C in moist chambers. After washing as above the sections were incubated with goat anti-rabbit IgG (bridge) antisera (Nordic, Sweden) at 1:20 dilution for 30 min at room temperature and again washed in PBS. The last incubation was made with rabbit PAP complex (Nordic, Sweden) at 1:50 dilution. Primary antisera and PAP complexes were diluted in PBS containing 2% BSA and 1% goat serum, in case of the "bridge" serum no goat serum was added. Bound rabbit anti-IgG was localized by staining with 0.05% 3,3′-diaminobenzidine and 0.01% hydrogenperoxide in 50 mM Tris-buffer, pH 7.6, for 5 min.

The following primary antisera were used: anti-human albumin, dilution 1:1000, 1:500; anti-human prealbumin, 1:500, 1:250; anti-human immunoglobulin, 1:1000, 1:500; anti-human Clq, 1:1000, 1:500; anti-human C3c, 1:1000, 1:500; and anti-human fibrinogen, 1:500. All antisera were from DAKO Immunoglobulins a/s, Denmark.

Results

Both in demented and non-demented aged controls, all serum proteins studied were shown to be present in the tissue around most large vessels in the white

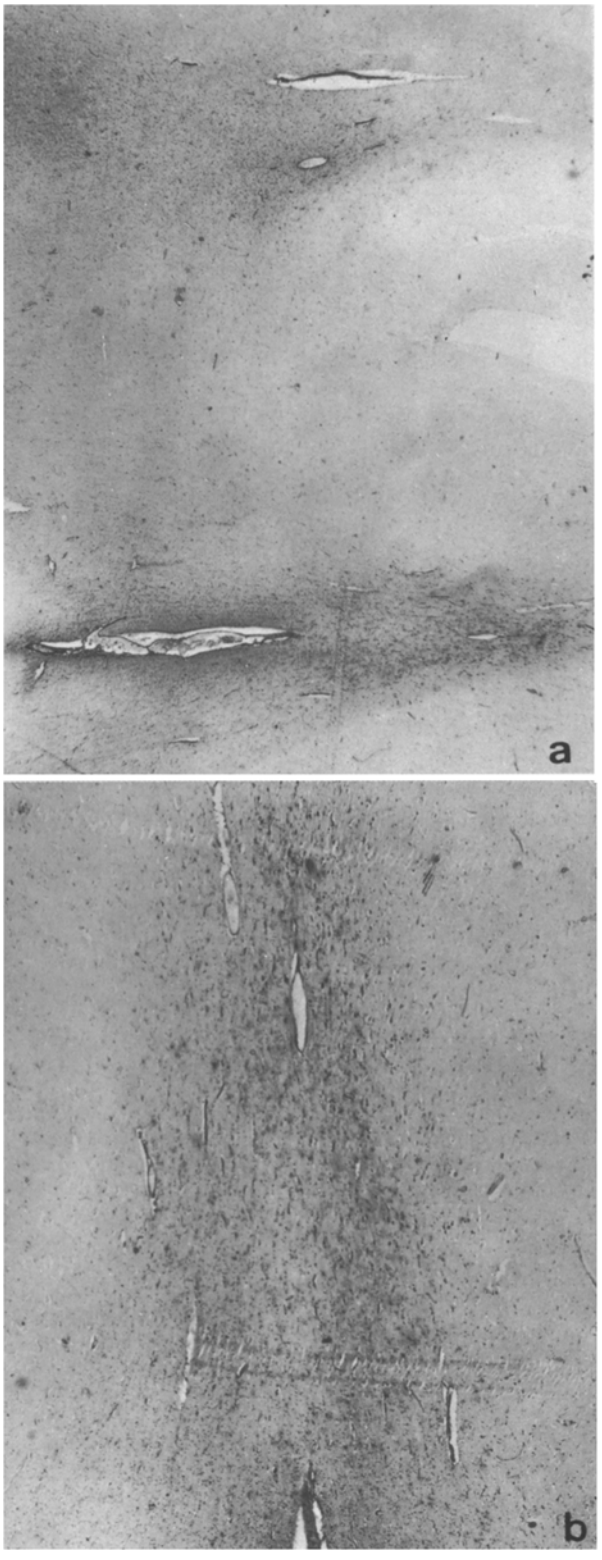

Fig. 1a, b. A section of frontal gyrus of a senile dementia of the Alzheimer type (SDAT) case stained with anti-IgG serum (1:500) showing physiological diffusion in the surroundings of the vessels of white matter. The tissue was from a 82-year-old male patient with clinical and histopathological diagnosis of SDAT (case no. 9), duration of the disease was 6 years. **a** ×10, **b** ×25

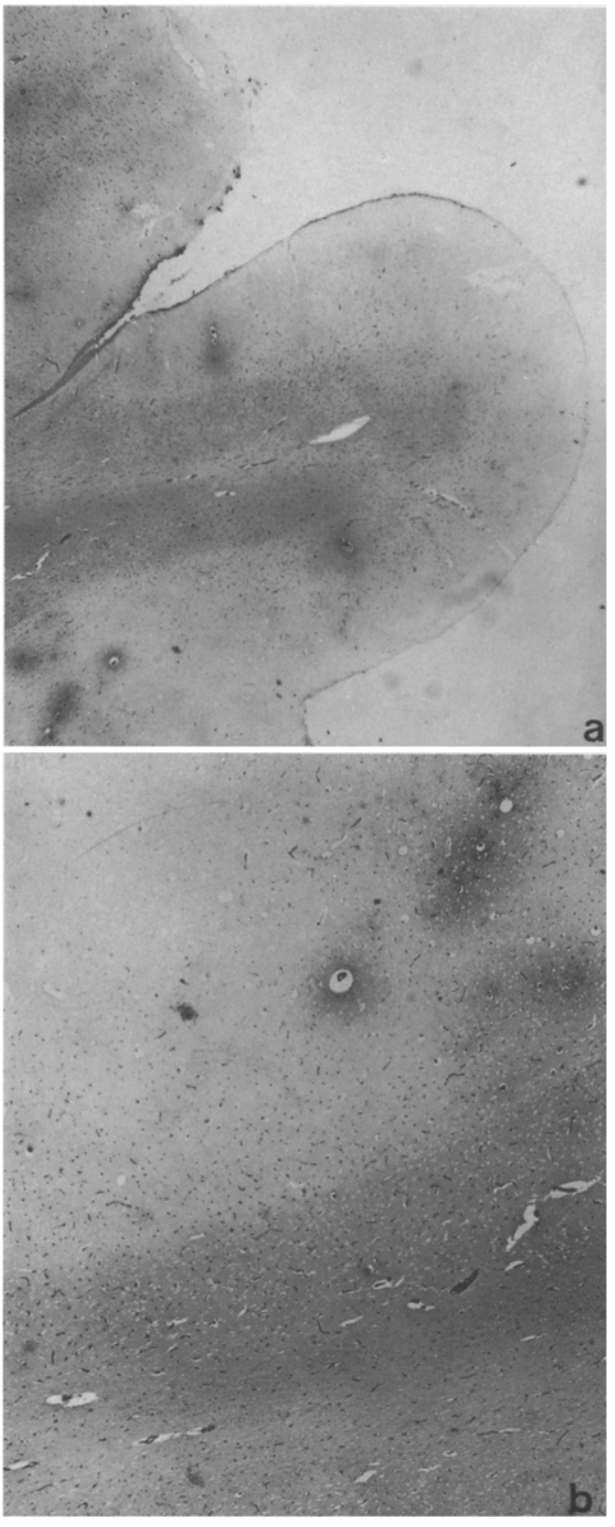

Fig. 2a, b. A section of frontal gyrus of a SDAT case stained with anti-IgG serum (1:500) showing vessels surrounded by the physiological diffusion in both grey and white matter. The section was from a 76-years-old male patient with clinical and histopathological diagnosis of SDAT (case no. 8), duration of the disease was 7 years. **a** ×10, **b** ×25

Fig. 3. A section of frontal gyrus of a SDAT case stained with anti-IgG serum (1:500). Neurons and glial are stained in the vicinity of the vessel. Diminishing staining of these cell structures with distance from the vessel. Same case as in Fig. 1; ×45

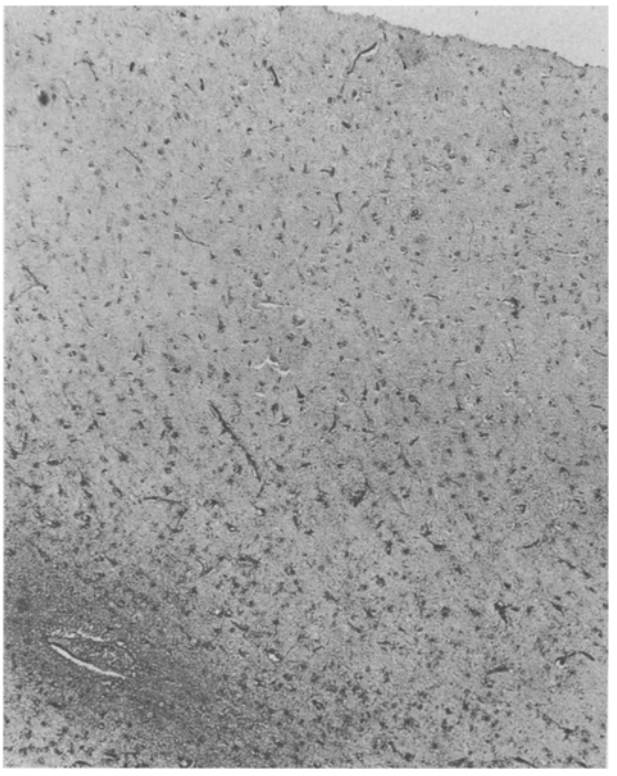

Fig. 4. A section from frontal cortex stained with anti-albumin sera (1:1000). The physiological diffusion in this non-demented case covers almost the whole section. The tissue was taken from a non-demented 67-year-old male individual. ×45

matter (Fig. 1). In one AD/SDAT case (case no. 8) perivascular staining with serum proteins was also observed around vessels in grey matter (Fig. 2).

The intensity of the staining gradually decreased with distance from the vessels. Most prominent staining was observed with antisera to albumin and IgG. Least extravascular staining was observed with antisera to prealbumin and fibrogen. The latter, however, darkly stained the lumen of the blood vessels. In demented cases, as well as in the normal aged controls, within the diffusion areas staining of neurons and glial cells was observed with the different antisera except with anti-fibrinogen. Occasionally staining of neurons and glial cells was also seen at such distance from the vessels that the neuropil was no longer stained (Fig. 3).

Variation in the intensity of staining of extravascular serum proteins was common not only with different antisera but also with same antisera in different fields in the same section as well as in different cases. Likewise the size of the diffusion area varied widely, ranging from small limited extravascular fields (Fig. 1) to covering almost the whole section (Fig. 4).

However, neither intensity nor size of the physiological diffusion correlated with histopathological diagnosis, age of the patient, cause of death or time between death and autopsy. In addition to the diffusion pattern, two of the MID/SDAT cases (case nos. 12 and 13) showed sharply demarcated darkly stained deposits in the surroundings of the capillaries of the grey matter; these serum protein deposits are frequently seen in cases with vasuclar dementia [3].

In both demented and non-demented individuals senile plaques, predominantly the plaque amyloid core (central core and whisps of amyloid) could be visualized occasionally with antisera to IgG, C1q and C3c (Fig. 5a–c). Although the immunostained plaques were generally located in or at the periphery of areas with increased perivascular diffusion, the staining of the plaques with these antisera was, in most of the cases, much stronger than that of the surrounding tissue (Fig. 5a–c). In a few instances only minimally stained plaques were observed, whereas the surrounding neurons were strongly immunostained. On the other hand, a rare immunostained plaque was seen in an area without detectable diffusion from any of the vessels (not shown).

Strikingly, no staining of plaques above background was observed with anti-albumin, anti-pre-

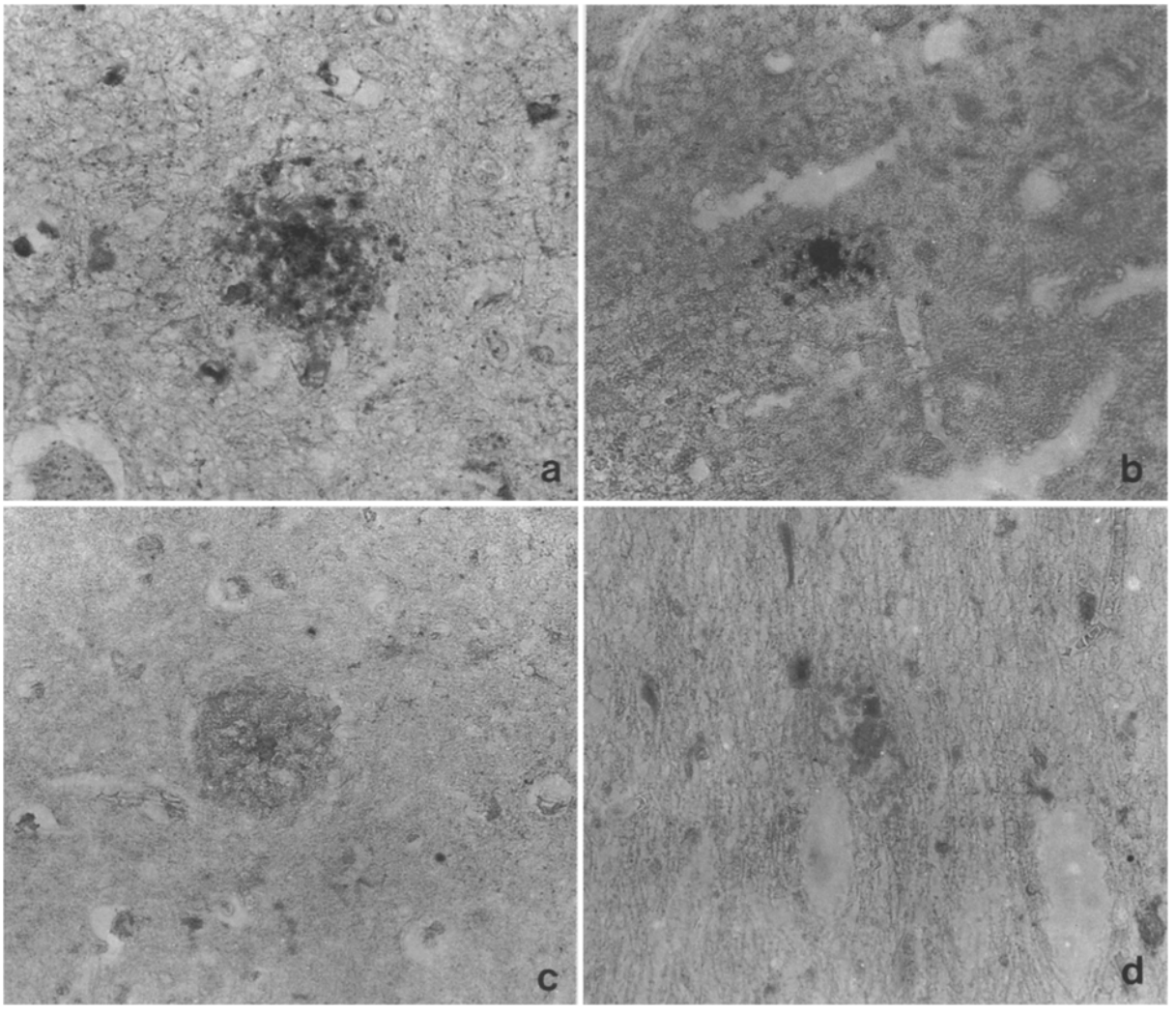

Fig. 5a–d. A section of frontal gyrus of a SDAT case stained with (**a**) anti-IgG serum (1:500), (**b**) anti-C1q serum (1:500), (**c**) anti-C3c serum (1:500) and (**d**) anti-prealbumin serum (1:500) Note the strong staining of the senile plaques in **a–c**, and the senile plaque hardly notable against the stained background in **d**. Same case as in Fig. 1; **a–d** ×300

albumin or anti-fibrinogen sera (Fig. 5d) despite the general staining of some areas of the section by the two former antisera. Neurofibrillary tangles were not visualized with any of the antisera studied.

Discussion

An altered BBB function in AD/SDAT [6, 18, 41] has been shown previously. The present study demonstrates that the BBB is compromised equally in AD/SDAT, MID/SDAT and in non-demented aged cases. In each of these three groups of cases there was a physiological diffusion of serum proteins into the surroundings of the vessels in white matter. There were no obvious differences in this diffusion, visualized with different antisera to serum proteins, between the demented and the non-demented aged individuals. A slight increase in immunoglobulin in CSF of normal elderly has been observed previously [20].

In both demented and normal aged individuals a broad spectrum of variation of staining intensity for the physiological diffusion was observed without any correlation to age, cause of death or time between death and autopsy. These differences may be due to the fact that the BBB function is labile, and is influenced by drugs [30], hypertension [16], epileptic seizures [1] and possibly by adrenergic activity [21]. The immunostaining of plaques with antisera to IgG and to complement components C1q and C3c, and the lack of reaction with antisera to other proteins studied suggest the presence of antigen/antibody complexes in plaques. This conclusion is supported by previous

studies which showed immunoglobulins [13, 17, 29] and complement factors in plaques in AD/SDAT [13].

The occurrence of immunoglobulins and complement in the plaques observed in this study and the localization of plaques in close relationship with vessel walls [23] suggest the possible implication of serum factors in development and/or maturation of plaques.

The immunological response, both cellular and humoral has been shown to decline with age [19, 27, 39, 44] and there seems to be a gradual upward trend in IgG and IgA in surviving individuals [10]. Important changes in the immunological system include the higher frequencies of autoantibodies and antinuclear antibodies in serum of the aged persons as compared to young ones [5, 11, 15, 39]. Autoantibodies directed to brain tissue, so called brain reactive antibodies (BRA), have been demonstrated in aged humans [26, 40] and mice [25]. The low concentrations of BRA in CSF and the lack of local production of antibodies in CSF in AD/SDAT patients [2, 18] support the contention that the serum might be the source of the IgG in the plaques.

It thus seems possible that etiology and maturation of plaques in normal aged and demented subjects may be due to a compromised BBB function and serum factors, while the etiology and maturation of neurofibrillary tangles may not be directly dependent on these factors.

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