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Complement activation in amyloid plaques in Alzheimer's disease brains does not proceed further than C3

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Abstract In Alzheimer's disease (AD) patients, the complement components Clq, C4 and C3 can be detected in different types of β/A4 plaques, one of the hallmarks of AD. Contradictory findings on the presence of late complement components in AD brains have been reported. Nevertheless, it was suggested in recent studies that in AD brain complement activation results in complement membrane attack complex (MAC) formation and that complement activation may act as an intermediate between β/A4 deposits and the neurotoxicity observed in AD. In the present study the presence of a number of complement components and regulatory proteins in AD temporal cortex and, for comparison, in glomerulonephritis (GN) was analysed. In GN kidneys, besides Clq, Clr, Cls and C3, the late components and the C5b–9 complex are also associated with capillary basement membrane and mesangial immune complex deposits. In AD temporal cortex Clq, C4 and C3 are co-localized with β/A4 deposits. However, in contrast to the GN kidney, the late complement components C5, C7 and C9, as well as the C5b–9 membrane attack complex cannot be detected in β/A4 positive plaques. The absence of the cytolytic C5b–9 complex in AD brain suggests that in AD, the complement MAC does not function as the proposed inflammatory mediator between β/A4 deposits and the neurofibrillary changes.

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Introduction

Alzheimer's disease (AD) is characterized by the presence of intraneuronal paired helical filaments and of extracellular amyloid deposits in senile plaques and cerebral vessel walls. Amyloid plaques in AD are mainly comprised of a 4 kDa β-amyloid peptide (β/A4) that is proteolytically derived from the transmembrane β-amyloid precursor protein and forms fibrillar aggregates [40]. With antisera specific for β/A4, two basic types of cerebral β/A4 plaques can be distinguished in AD brains. One type, in this study referred to as diffuse plaque, is characterized by diffuse, non-congophilic β/A4 deposits that are not associated with degenerating neurites and reactive glial cells. The other type consists of congophilic, fibrillar β/A4 deposits with glial and neuritic changes. A typical example of the latter is the classical amyloid plaque [6, 39]. We have shown in previous studies that classical pathway complement factors Clq, C4 and C3, but not the alternative pathway factor properdin, can be found in diffuse and classical plaques in the cerebral cortex and hippocampus of AD patients [7, 8, 39]. These complement factors are deposited as a result of an activation process, as was shown in earlier studies employing monoclonal antibodies specific for different epitopes exposed on C4 and C3 activation products [9, 11].

Activation of the classical pathway of complement can occur upon binding of the Clq recognition unit to an activator. IgG or IgM containing immune complexes, but also antibody-independent activation mechanisms, for example involving acute phase proteins, C-reactive protein or serum amyloid P, can initiate complement activation via the classical pathway (for reviews see [13, 41]).

It has recently been shown that Clq can interact with the β/A4 peptide in the absence of immunoglobulins and that β/A4 can activate the classical pathway of complement in vitro [20, 35, 36]. These findings offer a poten-

tial explanation as to why, even though no IgG or IgM can be detected in or around senile AD plaques [10, 35, 39], classical pathway activation of the complement system can occur in AD brains.

The immunocytochemical detection of the C5b–9 membrane attack complex (MAC) in AD brain dystrophic neurites and neurofibrillary tangles suggests that complement is involved in neuronal damage through the formation of this lytic complex [29]. It has been suggested that activation of the whole complement cascade may serve as an intermediate between β/A4 deposits and the neurotoxic effects observed in AD brains [22, 29, 35].

The lytic activity of MAC is regulated by several inhibitory proteins, including the fluid phase inhibitors vitronectin (S-protein) [18] and clusterin (Sp40,40) [19]. The demonstration of inhibitors of MAC formation (CD59, clusterin and vitronectin) in AD brain tissue supports the concept that complement activation leading to MAC formation contributes to AD pathology [28]. On the other hand, in diffuse plaques clusterin (Sp40,40), vitronectin and CD59 are present in the absence in the C5b–9 complex, which suggests that in the brain these inhibitors may serve another function than inhibition of MAC formation [47].

The presence of the early complement components Clq, C3, C4 has been confirmed by a number of research groups [15, 27, 29, 34, 35]. There are, however, still controversies about whether [29] or not [9, 16, 34] the late components that can form MAC are really present in AD brains.

We therefore decided to make a thorough investigation of the presence of various complement activation products and of the complement inhibitors Cl-esterase inhibitor (Cl-Inh), CD59, clusterin and vitronectin in AD-affected brain areas and in brain specimens from non-demented control cases. In addition, the presence of protease nexin 1 (PN1), a protease inhibitor that has been implicated in AD [37, 43], was investigated. As a positive control, renal biopsies manifesting various forms of glomerulonephritis (GN) in which, in additon to IgG or IgM deposits, Clq, C3 and the lytic MAC can be detected [2, 5, 32], were used.

Materials and methods

Tissue

Brain specimens from patients with senile dementia of the Alzheimer's type (SDAT) and from non-demented controls were obtained at autopsy within 3–9 h after death (brain bank in the Netherlands Institute for Brain Research Amsterdam; coordinator Dr. R. Ravid). Tissue blocks from the cerebral temporal cortex were snap-frozen and stored in liquid nitrogen until use. This study included six SDAT cases (ages 90, 83, 85, 90, 91, and 87 years). The clinical diagnosis of SDAT was neuropathologically confirmed on formalin-fixed, paraffin-embedded tissue from different sites. Many senile plaques, tangles and neuropil threads were present in the hippocampus and in the frontal, temporal and parietal neocortex, as demonstrated using Bodian and Congo red stains.

Five clinically non-demented controls (ages 75, 64, 68, 71 and 80 years) without clinical signs of any neurological disease were studied as controls. Neuropathological examinations revealed a few plaques and tangles in the hippocampus and in the temporal neocortex only. Samples from three patients (ages 75, 64, and 68 years) contained no or almost no β/A4 positive plaques, while in the other two cases (patients aged 71, and 80 years) many β/A4 deposits were present in the temporal cortex.

Kidney specimens were obtained at biopsy, snap frozen in liquid nitrogen and kept at –80° C unitl use. Four cases were studied: two IgA nephropathy cases showing mesangial IgA, IgM and Clq deposits, and two cases of diffuse proliferative GN (WHO class IV).

Immunohistochemistry

Frozen sections (5 μm) were mounted on poly-l-lysin-coated glass slides, air dried and fixed in cold acetone (10 min; RT). Sections were preincubated with normal swine serum (10% in phosphate-buffered saline [PBS]), followed by incubation (1 h; RT) with primary antibody in PBS containing 1% BSA. The primary antibodies used are listed in Table 1. Controls included incubation of the sections with non-immunized rabbit serum at a 1:50 dilution, or with PBS only, instead of primary antibodies. Prealbumin staining was performed to check for post-mortem vascular leakage.

Table 1 Monoclonal antibodies and antisera (*R* rabbit, *M* mouse, *ABC* biotinylated second antibody; avidin–biotin–peroxidase complex; DAB, *PAP* swine anti-rabbit 2nd antibody; HRP-rabbit anti-peroxidase complex; DAB, *ind. PO* indirect peroxidase technique)

Specificity	Source[a]	Most	Dilution	Technique
Clq	Zymed	R; IgG	1:50	PAP
Clr	Serotec	R; serum	1:100	PAP
Clr	CLB	R; serum	1:100	PAP
Cls	Serotec	R; serum	1:100	PAP
Cls	CLB	R; serum	1:100	PAP
Cl-Inh	Serotec	R; serum	1:100	PAP
Cl-Inh	v. Nostrand	R; serum	1:50	PAP
Cl-Inh	CLB; RII	M; mono	1:10	ABC
PN1	v. Nostrand	M; mono	1:100	ABC
C4c	DAKO	R; Ig	1:25	PAP
C3c	DAKO	R; Ig	1:100	PAP
C3d	CLB; 3–15	M; mono	1:50	ABC
C5	CLB	R; serum	1:100	PAP
C7	Quidel	M; mono	1:200	ABC
C7	Quidel	G; serum	1:200	ind. PO
C9	Quidel	M; mono	1:50	ABC
C9	Quidel	G; serum	1:50	ind. PO
C9	Morgan; 11.60	M; mono	1:100	ABC
C9	Morgan; 9.48	M; mono	1:100	ABC
MAC	Quidel	M; mono	1:50	ABC
MAC	DAKO: aE11	M; mono	1:50	ABC
MAC	Calbiochem	R; Ig	1:25	PAP
MAC	Morgan: B7	M; mono	1:75	ABC
Vitronectin	Quidel	M; mono	1:100	ABC
Sp40,40	Quidel	M; mono	1:20	ABC
CD59	Daha	R; serum	1:50	PAP
β/A4 (1–28)	Masters	R; IgG	1:300	PAP
Tau2	Sigma	M; mono	1:1000	ABC
Prealbumin	DAKO	R	1:500	PAP

[a] DAKO, Dakopatts, Glostrup, Denmark; CLB, Central Lab, Netherlands Red Cross Blood Transfusion Service, Amsterdam; Calbiochem, Calbiochem, San Diego,Calif.; Daha, gift from Dr. M.R. Daha, Nephrology, Department, Leiden University Hospital; Quidel, Quidel, San Diego,Calif.; Masters, gift from Dr. C.L. Masters, Pathology Department, University of Melbourne, Australia; Morgan, gift from Dr. B.P. Morgan, University of Wales College of Medicine, UK; Serotec, Serotec Ltd., Oxford, England; Sigma, Sigma, St. Louis, Mo.

Immunolabelling with mouse monoclonals was detected with biotinylated rabbit anti-mouse (DAKO, Denmark) and avidin–biotin–peroxidase complexes (ABC; Vector Laboratories, Calif.). Polyclonal antibodies were detected with either swine anti-rabbit second antibody (DAKO, Denmark) and peroxidase rabbit anti-peroxidase (PAP; DAKO, Denmark) complexes (rabbit antisera) or with biotinylated rabbit anti-goat antibodies (Sigma; St. Louis, Mo.) and streptavidin-HRP (Amersham, UK) (goat antisera). DAB was used as a chromogen. After immunoperoxidase staining, sections were counterstained with Congo red to visualize the amyloid.

Absorption experiments

Specificity of the anti-Cls and anti Cl-Inh antisera was tested in absorption experiments on GN kidneys and AD temporal cortex. Anti-Cls antisera were preincubated overnight at 4° C with serial dilutions (10–0.001 µg) of haemolytically active Cls per reaction mixture (100 µl) or buffer alone. After centrifugation (10000 *g*), the supernatant was applied to either kidney or brain cryostat sections. Similarly, anti-Cl-Inh antisera were absorbed with tenfold dilutions (100–0.01 µg) of purified Cl-Inh (Behring, Marburg, Germany).

Double immunostaining

After simultaneous incubation with polyclonal and monoclonal antibodies, mouse monoclonal antibodies were detected with biotinylated rabbit anti-mouse antibodies, ABC and DAB. Polyclonal rabbit immunoglobulins were detected with alkaline phosphatase conjugated goat anti-rabbit (Tago) antibodies and Naphtol AS Mx Phosphate and Fast Blue. Alternatively, the polyclonals were first visualized with swine anti-rabbit IgG, peroxidase anti-peroxidase complexes (PAP) and DAB, after which the mouse monoclonal antibodies were detected with rabbit anti-mouse antibodies, alkaline phosphatase mouse anti-alkaline phosphatase complexes (APAAP) and Naphtol AS Mx Phosphate and Fast Blue salt.

Estimation of the number of plaques

To gain an impression of the number of plaques in AD and controls, cryostat sections were reacted with antibodies specific for residues 1–28 of the β/A4 sequence. The number of β/A4 positive plaques was counted in representative areas, where the cortex was transversely cut. The viewing area of each microscopical field at the magnification used (×100) was 3.0 mm^2, measured with a gauged grid. The β/A4 deposits were classified according to Delaère et al. [6].

Results

With antiserum specific for β/A4 (1–28), temporal neocortex sections of two of the five non-demented controls showed high numbers (15–20 per mm^2) of mainly ill-demarcated β/A4 positive plaques. Only a few (1–3 per mm^2) β/A4 deposits with the appearance of classical plaques were observed. No staining for tau2 was observed in the neuropil or in the classical plaques of these controls.

In temporal neocortex sections of the AD patients included in this study many (15–20 per mm^2) β/A4-positive plaques were detected. These consisted of various types of β/A4 deposits according to the classification of Delaère et al. [6]. The most common were the diffuse plaques, but high numbers of well-demarcated plaques, including classical plaques, were also observed: 1–4 classical plaques per were seen. The well-demarcated plaques stained heavily for tau2, indicating neuritic changes.

Early complement components and inhibitors in temporal neocortex

Immune reactivity for the complement components Clq, C4 and C3 was detected in both diffuse and classical plaques (Table 2) in cerebral neocortex tissue from all six SDAT patients. No Cls immune reactivity was detected in either diffuse or classical plaques with the polyclonal anti-Cls (CLB). With another polyclonal anti-Cls (Serotec), a limited number of classical plaques were stained in four of the six cases. However, the immune reactivity of the Serotec anti-Cls on AD brain and GN kidney could not be adsorbed by preincubation with purified Cls. In double-labelling experiments with anti-Cls (CLB) and anti-β/A4, no Cls staining was observed in diffuse or classical β/A4 plaques. No Clr immune reactivity (Serotec anti-Clr) could be detected in the neuropil of 4 of the AD and of all control cases. In two AD cases faintly staining spherical structures were sporadically observed with both Serotec and CLB anti-Clr. Double labelling experiments with anti-Clr (CLB) and anti-β/A4 showed that in a very limited number (±1%) of diffuse β/A4 positive plaques weak Clr positivity was present.

No Cl-esterase inhibitor (Cl-Inh) immune reactivity could be detected in β/A4 plaques with three antibodies

Table 2 Complement components associated with β/A4 plaques in temporal cortex and in glomerulonephritis (GN) glomeruli, (*SDAT* senile dementia of Alzheimer type, *cl. pl.* classical plaques, *diff. pl.* diffuse plaques, + all cases positive staining, (–): some slight staining in some cases, – no positivity in any cases at all)

	SDAT cl. pl.	(*n*=6) diff. pl.	Control cl. pl.	(*n*=2) diff. pl.	GN kidney glomerulus
Clq	+	+	+	+	+
Clr	(–)	–	(–)	–	+
Cls	–	–	–	–	+
Cl-Inh	–	–	–	–	+
PN1	–	–	–	–	–
C4	+	+	+	+	+
C3	+	+	+	+	+
C5	–	–	–	–	+
C7	–	–	–	–	+
C9	–	–	–	–	+
MAC m; Q	+	–	–	–	+
MAC p; C	–	–	–	–	+
MAC m; D	–	–	–	–	+
MAC m; B7	–	–	–	–	+
vitronectin	+	+	+	+	+
Sp40,40	+	+	+	+	+
CD59	–	–	–	–	+
Rabbit serum control	–	–	–	–	–

tested. One of the antibodies used (Serotec rabbit anti-Cl-Inh) showed tangle, neuropil thread and vascular staining. This immunoreactivity could be absorbed only to a minor extent after preincubation of the antiserum with the highest concentration Cl-Inh used.

In addition, no immunostaining for protease nexin 1 (PN1), another inhibitor of complement proteases [42], was found in our around classical and diffuse plaques, except for one AD case in which PN1 immune reactivity was present in a few diffuse plaques.

Early complement components and inhibitors in GN kidney biopsies

In the kidney specimens exhibiting mesangioproliferative GN or diffuse proliferative GN, light to heavy mesangial and/or capillary basement membrane staining for Clq was observed. Glomerular staining restricted to the mesangium was also observed for the other subcomponents of Cl, Clr and Cls. No striking differences in staining were observed with the two polyclonal anti-Clr and the two polyclonal anti-Cls antibodies used. However, whereas it was possible for the anti-Cls (CLB) immunoreactivity on glomeruli to be adsorbed with 1–10 µg Cls/100 µl anti-Cls, the Serotec anti-Cls staining could not be inhibited, suggesting that the CLB antiserum has a higher specificity for Cls.

Mesangial staining for Cl-esterase inhibitor was observed in all four cases with the two polyclonal anti-Cl-Inh antisera (van Nostrand; Serotec) used. The immunoreactivity of one of the antisera (van Nostrand) with GN kidney sections could be adsorbed with 0.1–1 µg purified Cl-Inh. In addition, when a monoclonal anti-Cl-Inh (RII) was tested, two out of four cases showed mesangial staining. PN1 could not be detected in glomeruli of all kidney specimens tested, although tubuli were heavily stained.

Late complement components and inhibitors in temporal neocortex

In AD temporal cortex no C5, C7, C9 was found co-localized with either diffuse or classical plaques. Except for staining of some large blood vessels, no staining with either a polyclonal anti-C5 or any mono- or polyclonal anti-C7 or anti-C9 (Quidel) was observed in the neuropil. In addition, no staining was observed with two other monoclonal antibodies (9.48 and 11.60) specific for human C9 [31]. Different results were obtained when brain specimens were checked for the presence of C5b–9 with four different anti-MAC antibodies. Tangles, neuropil threads and dystrophic neurites within classical plaques stained with the Quidel monoclonal antibody. No staining pattern such as was observed with the Quidel monoclonal, and also no β/A4-associated staining, was seen with the three other anti-MAC antibodies (aE11 (DAKO) and B7 [23] monoclonals and the Calbiochem polyclonal). With none of the four anti-MAC antibodies tested was MAC staining observed in non-demented controls with diffuse and classical β/A4 positive plaques but without tau2 reactivity.

Clusterin and, to a lesser extent, vitronectin could readily be detected in both diffuse and classical plaques in both SDAT and control specimens.

CD59 could be detected in astrocytes and some neurons, but not within β/A4 deposits in both controls and SDAT.

Late complement components and inhibitors in GN kidney biopsies

In GN, as well as the early classical pathway components, C5, C7 and C9 were also detected in the glomerulus. In addition, identical staining patterns were observed with the four (B7, Quidel, DAKO and Calbiochem) anti-MAC antibodies used.

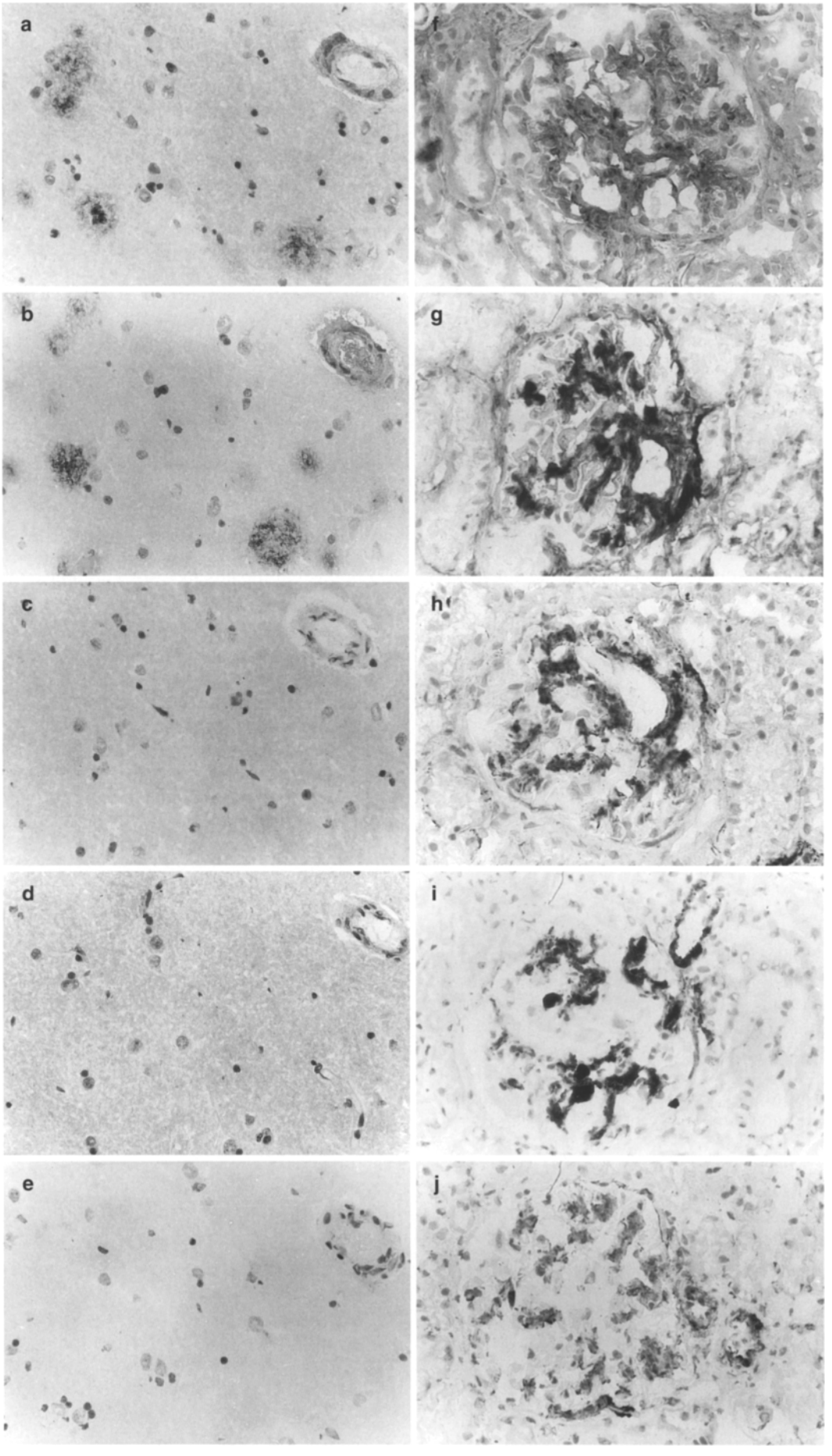
In all cases the fluid phase complement inhibitors clusterin and vitronectin, and also the membrane-associated CD59, showed a staining pattern similar to that observed for MAC.

Discussion

In AD cerebral cortex, the classical pathway components Clq, C4 and C3 colocalize with both classical and diffuse β/A4 plaques. No Clr, Cls or Cl-inhibitor (Cl-Inh), the only known inhibitor of Cl activation, was found associated with β/A4 deposits. Because some Clr reactivity is present in a very limited number of plaques in some cases, the possibility that Cl activation occurs in AD cannot be excluded. In general, Cl-Inh rapidly binds to the activated Cl, which leads to a dissociation of ClrCls(Cl-Inh)2 complexes from the activator-bound Clq. These complexes may either diffuse into the interstitial fluid or be taken up by phagocytic cells. On the other hand, the different staining results in AD brain and GN (Table 2) may be caused by much lower initial concentrations of the separate Cl subcomponents and also of Cl-Inh in the brain parenchyma than in the mesangium. The observed absence of Cl-Inh in the AD brain is in accordance with the Western blotting and ELISA results of other workers [36].

If high enough levels of the Cl subcomponents are present in the brain parenchyma to allow Cl assembly, a

Fig. 1a–e Adjacent sections of the temporal cortex of patient with Alzheimer's disease. Classical and diffuse plaques immunostain for C4c (**a**) and C3d, g (**b**). No C7 (**c**: Quidel monoclonal), C9 (**d**: Quidel monoclonal) or MAC (**e**: Calbiochem polyclonal) immunoreactivity is observed. ×400. **f–j** GN kidney sections immunostained for C4c (**f**), C3d,g (**g**), C5 (**h**), C7 (**i**: Quidel monoclonal) and MAC (**j**: Calbiochem polyclonal). Although negative on brain sections, anti-C5 (**h**), C7 (**i**) and MAC (**j**: Calbiochem) give mesangial staining on GN kidneys. ×400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total absence of Cl-Inh would allow unrestrained Cl autoactivation, resulting in classical pathway complement activation in the brain. Probably, the minute amounts of Cl-Inh in the brain are still high enough to maintain the approximately 2.5-fold molar excess of Cl-Inh over Cl that is needed to prevent spontaneous Cl activation in serum [48]. Alternatively, another protease inhibitor may control Cl activation in the brain parenchyma. In this connection, PN1, a brain-specific serine protease inhibitor [42], may have some role in the regulation of Cl activation in the brain. PN1 is best known as a potent antithrombin, but it also inhibits Cls and Clr, although not as effectively as Cl-Inh [42]. The involvement of PN1 in AD pathogenesis was first suggested when PN1-binding sites were found in plaques and tangles in AD brains [37].

Although PN1 is abundant in cortical tissue, especially in astrocytes [44], no PN1 immune reactivity could directly be detected in any of the plaques or tangles in cortical areas [37] (present study). However, protease-PN1 complexes, including Cls—PN1 complexes, are bound, rapidly internalized and degraded by cells [42]. Therefore, if Clr–PN1 complexes or Cls–PN1 complexes are formed in the brain they may be rapidly cleared, thus evading detection. It is noteworthy that the levels of PN1 in brain in AD are only one-sixth those in normals [43]. It is intriguing to speculate that the decreased levels of PN1 in AD brain may arise in part due to clearance of Clr–PN1 and Cls–PN1 complexes formed during early complement activation that occurs in this disorder.

In AD, MAC immunoreactivity, as detected with the Quidel monoclonal antibody, is confined to tangles, neuropil threads and dystrophic neurites and coincides with tau-related pathology [17, 29]. This finding, together with the observation that inhibitors of MAC formation are up-regulated in AD brains, led to the concept that MAC formation is contributing to AD pathology [28, 29]. However, except with the Quidel monoclonal anti-MAC used in these studies, we could not detect MAC in AD temporal cortex. In addition, no immunostaining for the late components was detected in brain specimens, whereas in GN, as well as the early classical pathway components, C5, C7, C9 and MAC were also detected in glomeruli (Table 2, Fig. 1). Two non-demented controls with diffuse and classical β/A4-positive plaques, but without tau2 positivity, did not react with the Quidel anti-MAC. These findings, together with the observed cytoplasmatic staining of tangles with the Quidel anti-MAC, and the inability to obtain tau-related MAC staining in AD with three other antibodies, suggest that the Quidel anti-C5b–9 cross-reacts with components of paired helical filaments, which are found in neuropil threads and neurofibrillary tangles. Differences in specificity of the anti-C5b–9 antibodies used may be due to the use of different immunization and screening protocols. The Quidel anti-C5b–9 monoclonal antibody was obtained from fusions of spleen cells from mice hyperimmunized with soluble C5b–9 complexes, whereas the other antibodies used were obtained after immunization with polymerized poly-C9 (B7: [23]) or with purified membrane-bound C5b–9 complexes obtained from complement lysed cells (Calbiochem polyclonal: [3]; DAKO a E11: [30]).

CD59 is the most important inhibitor of C5b–9 membrane insertion to protect cells from bystander lysis [24]. Except on astrocytes, no CD59 can be found in the brain parenchyma of AD and controls (present study) [47]. This finding, together with the observation that no late complement components are present in the brain parenchyma, suggests that a C5b–9 initiated active debris (e.g. dystrophic neurites) clearance does not play an important role in AD. In AD brains clusterin and vitronectin can be detected in nearly all diffuse and classical plaques [47]. Their presence at sites where no complement activation further than C3 occurs suggests that clusterin and vitronectin in the brain serve some other function than complement regulation. Clusterin possibly plays a role in cell–extracellular matrix interaction during synaptic remodelling [19, 26]. Similarly, vitronectin–vitronectin receptor interactions may allow cell attachment during tissue regeneration [4].

Our findings that no complement activation products further than C3b can be detected in AD brains are at odds with the current idea that complement activation in AD brains culminates in C5b–9 complex formation with subsequent neurotoxicity [29, 35, 36]. The function of the early complement components remains to be investigated. Brain-derived cells are able to produce various complement components in vitro [12, 45]. RNA in situ hybridization experiments have shown that Clq, C4 and C3 and the regulatory protein clusterin are locally produced in AD brain [21] and in experimentally deafferentiated rat brains [33]. ClqB mRNA co-localized with CR3-positive cells [33], which suggests that activated microglia are probably the main producers of Clq.

The increased local production of the early complement components in AD may serve different purposes:

1. To influence the size and complexity of amyloid fibrils. In a manner reminiscent of the complement-mediated solubilization of immune complexes after incorporation of C3b–iC3b [1], activated C3 may reduce the size of amyloid deposits. On the other hand, Clq can bind to β/A4 [20, 35, 36] and can accelerate β/A4 aggregation in vitro [46].
2. To opsonize β/A4 deposits or dystrophic neuronal elements. Deposited Clq or (i)C3b may interact with phagocytic cells via either Clq or iC3b receptors. Activated microglial cells (HLA-DR$^+$; CR3$^+$, CR4$^+$) displaying iC3b receptors can be found in and around classical plaques [10, 38].
3. Locally produced Clq may regulate the glial response in AD-affected areas of the brain, in the same way as it has anti-proliferative [14] and stimulatory effects [25] on macrophages in vitro.

Our present findings suggest that in AD, high enough levels of the early components C1, C4, C3, are present to allow the complement pathway to proceed until C3. C1 through C3 can be found associated with β/A4 deposits in both AD and controls, irrespective of the presence or

absence of tau pathology or activated glial cells. The late components (C5, C7, C9) are below detection levels or absent, so that no lytic complex can be formed. In contrast to current opinion, this indicates that in AD, complement does not function as an inflammatory mediator involved in cytotoxicity via the C5b–9 complex formation. The function of the locally produced Clq, C4 and clusterin in AD, and also after experimental brain lesioning, still remains unclear. Therefore, further studies are needed to determine what role complement factors have in the brain.

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