

## Significance of Complement Activation in Alzheimer's Disease

a report by

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Complement activation is a major inflammatory process whose primary functions are to assist in removing micro-organisms and cellular debris and processing of immune complexes. The complement system is composed of more than 30 plasma and membrane-associated proteins, accounting for approximately 10% of the globulins in vertebrate serum, which function as an inflammatory cascade. Complement can be activated by many factors, including immune complexes, polysaccharides (including lipopolysaccharide, the major component of the outer membrane of Gram-negative bacteria), and neuropathological structures such as senile plaques, neurofibrillary tangles (NFTs), and Lewy bodies.<sup>1-4</sup>

When activation of the system occurs, native complement proteins are enzymatically cleaved, generating complement 'activation proteins' that function as opsinins, anaphylatoxins, and chemokines (see *Table 1*). The liver is the main source of the complement proteins in peripheral blood, but these proteins are also produced in other tissues and organs, including the brain.<sup>5</sup> In the central nervous system (CNS), complement proteins are synthesized by a variety of cells including neurons, microglia, astrocytes, oligodendrocytes, and endothelial cells.<sup>5</sup> Three complement pathways, the classic, alternative, and lectin-mediated cascades, have been identified (see *Figure 1*). These pathways differ in the mechanisms that activate them, but full activation of any of the pathways produces C5b-9, the 'membrane attack complex' (MAC). The MAC penetrates the surface membrane of susceptible cells on which it is deposited, and can result in cell lysis if present in sufficient concentrations;<sup>6</sup> conversely, sublytic concentrations of the MAC can be protective on some types of cell.<sup>7</sup> Complement activation is normally closely regulated through the actions of endogenous complement inhibitory proteins,<sup>8</sup> including CD59, clusterin, vitronectin, C1-inhibitor, complement inhibitor C4b-binding protein, decay-activating factor, and factor H. When these regulatory mechanisms are insufficient, then tissue damage can result. Because

complement activation exerts both protective and deleterious effects, it has been referred to as a 'double-edged sword.'<sup>1</sup>

### Status of Complement Activation in the Alzheimer's Disease Brain

C1q (the first protein in the classic complement pathway), early complement activation proteins (C4 and C3 activation fragments), and the MAC have been demonstrated by immunocytochemical staining in the Alzheimer's disease (AD) brain on senile plaques, NFTs, neuropil threads, and dystrophic neurites<sup>9-12</sup> (see *Figure 2*). Increased mRNA levels for native complement proteins are also present.<sup>13</sup> Complement activation is thought to be triggered in the AD brain primarily by the interaction of complement proteins with aggregated forms of amyloid-beta (A $\beta$ ) and tau protein, the major components in plaques and NFTs, respectively.<sup>2,3,12</sup> Soluble, non-fibrillar A $\beta$  may also be capable of activating complement, albeit to less of an extent than fibrillar A $\beta$ .<sup>14</sup> Complement activation and plaque formation are mutually promoting mechanisms. Aggregated A $\beta$  efficiently binds C1q, activating the classic complement pathway,<sup>2</sup> and this process further enhances A $\beta$  aggregation and fibril formation.<sup>15</sup> Whether elevated complement activation in AD may result, in part, from impaired local defense mechanisms is not clear, due to conflicting reports about the status of complement inhibitory proteins in the AD brain.<sup>16,17</sup>

Complement activation in AD was initially reported to be limited to the classic pathway,<sup>9</sup> but alternative pathway activation was later reported as well.<sup>18</sup> The significance of complement activation in the development and progression of AD is unclear. Several of the activation proteins generated in this process have been demonstrated to exert neuroprotective effects *in vitro*, including protecting against excitotoxicity,<sup>19</sup> A $\beta$ -induced neurotoxicity,<sup>20</sup> and apoptosis,<sup>7</sup> as well as facilitating the clearance of A $\beta$  by microglia.<sup>21</sup> In contrast, the MAC is toxic to neurons on which it is deposited,<sup>6</sup> and also to adjacent neurons via 'bystander lysis.'<sup>22</sup> Activation of complement can also enhance other neurotoxic processes in the AD brain: it increases A $\beta$  aggregation<sup>15,23</sup> and potentiates its neurotoxicity,<sup>24</sup> and it attracts microglia<sup>25</sup> and promotes their secretion of inflammatory cytokines.<sup>26</sup> The close association of complement staining, particularly the MAC, with pathological structures in the AD brain suggests that complement activation may contribute to the neurodegenerative process in AD, despite the neuroprotective actions of some complement proteins. Indeed, this process was generally accepted to play a deleterious role in AD until the publication in 2002 of a study by Wyss-Coray, et al.<sup>27</sup> An animal model of AD, the transgenic APP mouse, was crossed with mice expressing soluble complement receptor-related protein y (sCrry), a rodent-specific



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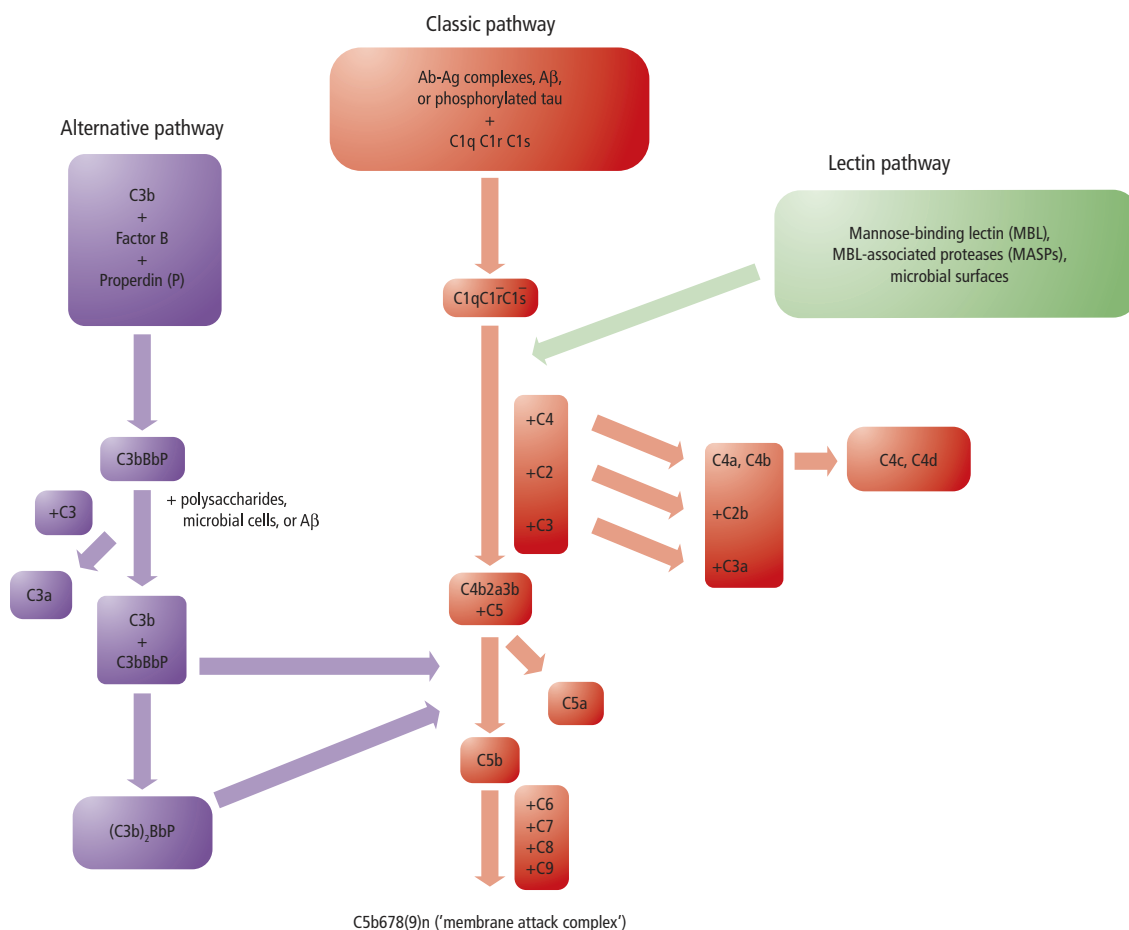
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**Table 1: Biological Activities of Complement Proteins with Relevance to Alzheimer's Disease**

Name	Biological Activity
C1q	Enhances A $\beta$ aggregation; may facilitate A $\beta$ clearance; enhances A $\beta$ -induced cytokine secretion by microglia
C3a	Anaphylatoxin (increases capillary permeability); protects neurons from excitotoxicity
C3b	Immune adherence and opsonization (may facilitate A $\beta$ clearance by phagocytic microglia)
C4a	Anaphylatoxin (weak)
C5a	Anaphylatoxin; protects neurons from excitotoxicity; chemotactic attraction of microglia; inhibits apoptosis; increases cytokine release from A $\beta$ -primed monocytes
C5b-9	Neurotoxicity; sub-lytic concentrations may have both pro- and anti-inflammatory activities

Source: Loeffler, J Neuroinflamm, 2004;1:18.

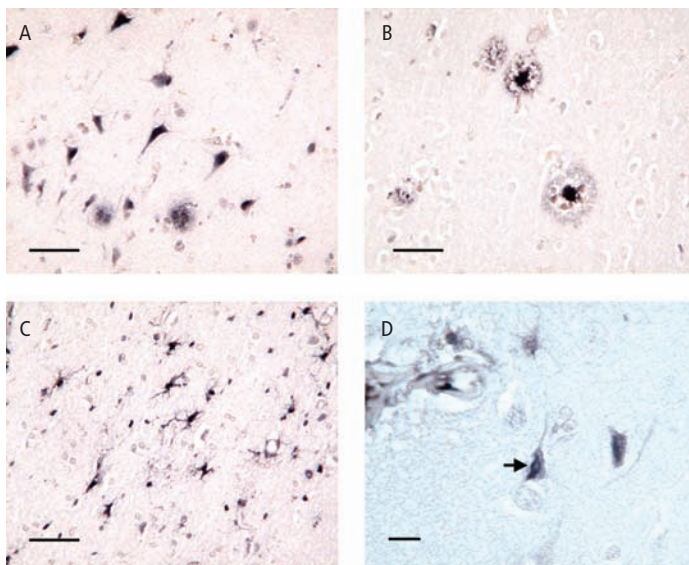
**Figure 1: Schematic Diagram of the Three Complement Activation Pathways**

Complete activation of any of these three pathways generates the neurotoxic membrane attack complex (MAC), C5b-9. Source: Loeffler, J Neuroinflamm, 2004;1:18.

inhibitor of early complement activation. The APP/sCrry mice had a two- to three-fold increase in cortical and hippocampal A $\beta$  deposition, together with a 50% loss of pyramidal neurons in region CA3 of the hippocampus. The authors concluded that complement activation may protect against A $\beta$ -induced neurotoxicity. Some subsequent investigations in animal models of AD have also suggested a neuroprotective role for complement in AD; Maier et al.,<sup>28</sup> using C3-deficient APP mice, found a beneficial role for C3 in plaque clearance, and Zhou et al.<sup>29</sup> found evidence for both detrimental and protective effects of complement in APPQ<sup>-/-</sup> (C1q-deficient) mice. However, Pillay et al.<sup>30</sup> found that intracranial administration of vaccinia virus complement control protein, which inhibits both the classic and alternative

complement pathways, significantly reduced memory deficits in APP mice. The relevance of these findings to AD is unclear, because full activation of complement has not been reported in the APP mouse. One reason for this difference from the AD brain is that mouse C1q binds less efficiently than human C1q to human A $\beta$ , resulting in less activation of mouse complement by the human A $\beta$  present in plaques in the APP mouse.<sup>31</sup> Lack of appropriate anti-sera for detecting the mouse MAC may be an additional reason why the MAC has not been detected in these animals. Thus, because the main neurotoxic component of complement activation, the MAC, is apparently lacking in these mice, the balance between complement's neuroprotective and neurotoxic effects may be different in APP mice than in AD patients.

**Figure 2: Complement Immunoreactivity in Plaques, Neurons, and Neurofibrillary Tangles from the Inferior Temporal Cortex**



A: iC3b-stained plaques and neurons, mild cognitive impairment (MCI) specimen; B: C9-stained plaques, AD specimen; C: iC3b-stained glial cells, aged normal specimen; D: iC3b staining of possible neurofibrillary tangle, AD specimen. A–C: bar = 50 $\mu$ m; D: bar = 12.5 $\mu$ m. Source: Loeffler, et al., *J Neuroinflamm*, 2008;5:9.

### The Role of Complement Activation in the Development of Mild Cognitive Impairment and/or Early Alzheimer's Disease

Complement activation in the brain has been examined in 'high pathology controls' (non-demented elderly subjects who are found, on *post mortem* examination, to have extensive AD-type brain pathology) and in subjects with mild cognitive impairment (MCI), a transitional state between the cognitive levels in normal (non-cognitively impaired) aged subjects and those with dementia. Plaque-associated MAC staining was only slightly increased in the frontal gyrus in these individuals and was far less than in AD patients, although total plaque numbers were similar between the two groups.<sup>32</sup> Zanjani et al.<sup>33</sup> subsequently found that early complement activation (C4d) on plaques in the temporal cortex increased in association with total plaque numbers from very mild to severe clinical AD. The MAC was detected on plaques and NFTs in some very mild cases but consistently only in severe AD. A recent study from the author's laboratory<sup>34</sup> measured plaque-associated complement staining in the inferior temporal cortex from aged normal, MCI, and AD patients. Early-stage (iC3b) and late-stage (C9) complement activation was found in low numbers on plaques in both aged normal individuals and subjects with MCI; total and complement-stained plaque numbers did not differ between these two groups and were 2.5- to three-fold less than in subjects with AD. Plaque complement staining was highly correlated with total plaque counts, and both of these parameters were inversely associated with measures of cognition. The authors performed regression analysis in an effort to determine the extent to which plaque-associated complement activation might contribute to cognitive deficits, but the analysis failed because of the strong correlation ('multicollinearity') between total plaque counts and complement-stained plaques. So, although complement activation increases in parallel with plaque counts during clinical progression from normal cognition (in aged subjects) to AD dementia, its role in this cognitive loss remains unclear.

### Why A Determination of the Role of Complement Activation in Alzheimer's Disease Is Important

AD is the most common form of dementia, accounting for 60–80% of all cases. According to the Alzheimer's Association,<sup>35</sup> approximately 5.2 million Americans suffer from this disease and, by 2030, the number of Americans aged 65 and over with AD is expected to be approximately 7.7 million. The drugs currently approved by the US Food and Drug Administration (FDA) for treatment of AD do not slow progression of the underlying neuropathology, although they provide short-term symptomatic relief to some patients. If complement activation does play an important role in the development and/or progression of this disease, reducing this process in the brain could produce a major breakthrough in the treatment of AD. Some complement-inhibiting drugs are already available and others are being developed.<sup>36</sup> Selectively inhibiting late-stage activation, which produces the neurotoxic MAC, would seem to be a logical approach for inhibiting complement in AD patients. Eculizumab (Soliris, Alexion Pharmaceuticals),<sup>37</sup> a humanized anti-C5 monoclonal antibody that prevents C5 cleavage (and, therefore, formation of the MAC) could be considered for a small clinical trial in patients with mild AD. This drug was recently used successfully in a phase III trial in patients with paroxysmal nocturnal hemoglobinuria.<sup>38</sup> The extent to which this drug crosses the blood–brain barrier is unknown; however, permeability of the blood–brain barrier to systemically administered antibodies may not be a problem in AD patients, as suggested by two recent clinical trials in which systemic treatment with intravenous immunoglobulins resulted in improvement in cognitive scores.<sup>39,40</sup>

Complement activation is a major inflammatory process whose primary functions are to assist in removing microorganisms and cellular debris and processing of immune complexes.

### Conclusions

- Complement activation is a major inflammatory process that co-localizes with neuropathological structures in the AD brain, and progressively increases as this pathology becomes more extensive. It does not appear to be increased in the brains of individuals with MCI, however.
- Despite extensive investigation, the significance of this process in the development and progression of AD is unclear, because it exerts both neuroprotective and neurotoxic effects *in vitro*. Studies in animal models of AD have not succeeded in resolving this problem.
- A clinical trial in which a selective inhibitor of late-stage complement activation would be administered to AD patients should be considered. ■

### Acknowledgment

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## Editor's Recommendation

### Plaque Complement Activation and Cognitive Loss in Alzheimer's Disease Loeffler D, et al.

*J Neuroinflamm*, 2008;5:9.

The objective of this study was to determine the relationship between complement activation and cognition during the development of Alzheimer's disease (AD). iC3b, C9, Bielschowsky, and Gallyas staining was performed on aged normal (n=17), mild cognitively impaired (n=12), and AD (n=17–18) inferior temporal gyrus specimens. Plaques were counted in 10x fields with high numbers of Bielschowsky-stained plaques. One-way ANOVA was used to determine between-group differences for plaque counts and measures of cognitive function, and linear regression was used to evaluate global cognition as a function of Bielschowsky-stained plaques. Terms for iC3b- and C9-stained plaques were then added sequentially as additional predictors in a 'mediation analysis' model. Complement was detected on plaques in all groups, and on neurofibrillary tangles only in AD specimens. iC3b, C9, and Bielschowsky-stained plaque counts increased 2.5- to three-

fold in AD versus other groups (all  $p \leq 0.01$ ). C9 staining was present on some diffuse plaques, and as on neuritic plaques. Bielschowsky-stained and complement-stained plaque counts were highly correlated, and were negatively correlated with cognitive measures. When the Bielschowsky plaque count was used as a predictor, its correlations with cognitive measures were statistically significant, but when iC3b and C9 plaque counts were added as additional predictors, these correlations were not significant. This was attributed to multicollinearity, i.e. both early-stage (iC3b) and late-stage (C9) complement activation occurs on neocortical plaques in subjects across the cognitive spectrum; contrary to previous reports, C9 is present on some diffuse plaques. Because of high correlations between complement-stained and Bielschowsky-stained plaque counts, quantitative assessment of the extent to which complement activation mediates the relationship between plaques and cognitive function could not be performed. Additional studies with animal models of AD (with late-stage complement activation), or a trial in AD patients with an inhibitor of late-stage complement activation, may be necessary to determine the significance of this process. ■