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# Role of Activated Glia and of Glial Cytokines in Alzheimer's Disease: A Review

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## SUMMARY

We review the role of activated microglia and activated astrocytes, and of glia-derived cytokines and other molecules, in the pathogenesis and pathophysiology of Alzheimer's disease. Activated microglia overexpressing the potent immune-response cytokine interleukin-1, and activated astrocytes over-expressing the neurotrophic cytokine S100β, are near-constant components of neuritic plaques in Alzheimer's disease, and are frequent components of early, non-neuritic, amyloid deposits. The known biological activities of these two cytokines suggest an orchestrating effect on the complex cellular and molecular interactions that drive plaque progression. Furthermore, findings in brains of patients dying with conditions known to predispose to Alzheimer's disease suggest that activation of glia and overexpression of glial cytokines are early events in Alzheimer pathogenesis. These results suggest that therapeutic intervention directed toward interrupting the driving immunological processes in Alzheimer's disease might slow or arrest progression of clinical disease.

## INTRODUCTION

Glia have attracted increasing attention for their role in both normal and pathological brain functioning. Two glial cell types in particular, microglia and astrocytes, are now known to actively respond to neural injury through proliferation, migration, hypertrophy, and elaboration of cytokines—secreted molecules that signal adjacent cells to undergo morphological and metabolic alterations of their own. These cell types have long been recognized as cells that 'react' to neuronal injury, but recent work has extended our understanding beyond such 'reactive' roles, and microglia and astrocytes are now known to participate in many inflammatory and neuromodulatory actions. In light of such 'active' functions, migroglia and astrocytes responding to neuronal alterations and injury are now more properly referred to as 'activated'. Microglia are rather nondescript cells in routine histological preparations of brain, and this situation is not improved by ultrastructural examination (1). Our knowledge of microglia has benefitted greatly from the relatively recent introduction of specific lectins and immunohistochemical reagents that facilitate their visualization and identification. Moreover, it is through this latter technique—immunohistochemistry—that one can now identify the precise cellular site, and extent of expression, of important biological molecules such as the potent immune-response cytokine interleukin-1 (IL-1). Our early demonstration that activated microglia, overexpressing IL-1, are intimately associated with the diagnostic neuritic plaques of Alzheimer's disease (2) immediately suggested an active immune, or inflammatory, component in the pathophysiology of these lesions and, by extension, in Alzheimer's disease itself. Susequent work has suggested that microglia-derived IL-1 not only *participates* in the evolution of Alzheimer lesions, but that this cytokine is a key *orchestrating* factor in plaque evolution. It also suggests that IL-1 attracts activated astrocytes to these lesions and induces (both directly and indirectly) formation of the overgrown neuritic processes that characterize these diagnostic Alzheimer lesions.

Astrocytes are now known to participate in many neu-romodulatory actions through control of local ionic and neurochemical concentrations, synthesis and release of biologically important molecules, and intercellular signalling through chemical messengers (cytokines). Activated astrocytes, like activated microglia, are near-universal components of the neuritic plaques of Alzheimer's disease (2,3,4), and activated astrocytes elaborate a variety of molecules that are important in Alzheimer's disease. These include $\alpha_1$-antichymotrypsin (5), apolipoprotein E (5) and the complement protein C3 (6) — all of which are components of the extracellular protein deposits of neuritic plaques (7,8,9,10) — and the neuroactive cytokine S100$\beta$ (2), which manifests trophic influences on neurons, on neurites, and on other astrocytes.

## ACTIVATED GLIA IN THE PLAQUES OF ALZHEIMER'S DISEASE

Neuritic plaques in Alzheimer's disease are complex structures. In addition to the swollen, abnormal neuritic growths that are the basis for their identification, they invariably contain extracellular protein deposits, rich in an amyloidogenic protein fragment that condenses into a congophilic, $\beta$-pleated sheet form recognized histologically as amyloid. This amyloid may be finely dispersed throughout the plaque (a *diffuse neuritic plaque),* or may be present as one or more dense core deposits surrounded by finely dispersed amyloid (a *dense core neuritic plaque).* As mentioned above, a number of other proteins have been identified in these deposits. In addition— as suggested above—there is a glial component to neuritic plaques, composed of microglia and astrocytes. Plaque-associated microglia are found at the center of virtually all (>97%) neuritic plaques (11). These microglia are activated and overexpress IL-1 (11). Activated astrocytes, overexpressing S100$\beta$, are also found associated with virtually all neuritic plaques in Alzheimer's disease (4), but in contrast to activated microglia, plaque-associated activated astrocytes are found at the periphery of the plaque, as a spherical shell encasing the entire structure (4). From this peripheral location, these astrocytes extend processes deep into the plaque, where they appear to contact both the neuritic elements and the microglial cells.

Neuritic plaques are thought to evolve from putative precursor lesions consisting of diffuse amyloid deposits, devoid of ot condensed congophilic $\beta$-pleated amyloid and neuritic elements. These lesions are found concomitant with neuritic plaques in brain of Alzheimer patients, but may also be found occasionally in elderly patients without significant numbers of neuritic plaques and without clinical evidence of cognitive decline (12,13). This latter observation suggests that these *diffuse non-neuritic plaques* are not, in and of themselves, injurious to brain, rather it is the progression of these structures into neuritic plaque forms that, although apparently not inevitable, produces clinical disease. However, the precise trigger or triggers that initiate plaque progression remain obscure. Indeed, the precise trigger or triggers that initiate amyloid deposition in the first place are still to be identified. We do know that the trigger for plaque progression is probably some biochemical or biophysical alteration in the amyloid deposit that confers immunogenicity upon the deposited protein. This is based on our observation that, in Alzheimer's disease, the great majority (78%) of diffuse non-neuritic plaques contain both activated microglia, overexpressing IL-1 (11), and activated astrocytes, overexpressing S100$\beta$ (14). Following initiation of dystrophic neurite growth in amyloid plaques, these structures are thought to progress through three additional morphologically identifiable stages (15): the previously mentioned diffuse neuritic and dense core neuritic plaques, and the final, end-stage *dense core non-neuritic plaque,* characterized by a solitary dense core of condensed $\beta$-pleated amyloid, devoid of diffuse amyloid and of neuritic elements. Each of these plaque types has a distinct pattern of associated activated glia (11,14): the small numbers of activated microglia and astrocytes associated with diffuse non-neuritic plaques increase to maximal numbers at the diffuse neuritic plaque stage. There is then a waning in numbers of these glia in the dense core

neuritic plaque stage, and a decrease to nearly zero in the numbers of such glia associated with dense core non-neuritic plaques.

## GLIAL CYTOKINES IN PLAQUES OF ALZHEIMER'S DISEASE

The pattern of association between activated glia and postulated stages of plaque evolution suggests a waxing and waning of a glial inflammatory response with plaque progression. Moreover, the known trophic and toxic effects of biologically active molecules elaborated by these plaque-associated glia allow a mechanistic interpretation of this pattern, lnterleukin-1, elaborated early in plaque evolution by activated microglia associated with diffuse non-neuritic plaques, promotes synthesis (16) and processing (17) of $\beta$-amyloid precursor protein ($\beta$-APP), a normal membrane protein that can be cleaved to form either the amyloidogenic peptide mentioned above, or to form alternative neurotrophic fragments (18). IL-1 may thus have a role in promoting further amyloid production and deposition in plaques. IL-1 activates astrocytes (19) and may be responsible for attracting the spherical shell of astrocytes that encircle neuritic plaques. IL-1 has also been shown to induce astrocytic expression of $\alpha_1$-antichymotrypsin (5), apolipoprotein E (5), and the complement protein C3 (20) which, as mentioned above, are all found within the amyloid deposits of neuritic plaques in Alzheimer's disease (7–10). IL-1 also induces overexpression of the astrocytic cytokine S100$\beta$ (21), which itself may participate in the pathophysiological dynamics of plaque formation and evolution. Tissue levels of S100$\beta$ are elevated in brains of Alzheimer patients (22), and the S100$\beta$ extracted from such tissue is biologically active (22). S100$\beta$ is a neurite growth-promoting factor (23), which has been implicated in promoting the growh of abnormal, swollen ("dystrophic") neurites that characterize neuritic plaques (4). These dystrophic neurites overexpress $\beta$-APP (11), a result that may be attributable in part to the $\beta$-APP-upregulating properties of IL-1 mentioned above. This proposed role of S100$\beta$ in dystrophic neurite formation is supported by our demonstration of a significant correlation between the cross sectional area of $\beta$-APP immunoreactive neurites and the number of plaque-associated S100$\beta$ immunoreactive astrocytes within neuritic plaques in Alzheimer's disease (14).

IL-1 and S100$\beta$ thus appear to be key components of a cascade—initiated by microglial overexpression of IL-1 in diffuse non—neuritic amyloid plaques—that culminates in formation of neuritic plaques. The initial events of this cascade presumably represent 'normal' reparative responses to tissue injury, which are carried to damaging extremes in the plaques of Alzheimer's disease. This interpretation is analogous to the recognized damaging effects of excessive inflammatory responses in peripheral degenerative diseases. This idea suggests that elements of such a cascade might be present in various acute and chronic neurological insults, and in fact this is so. Expression of IL-1 by microglia and of S100$\beta$ by astrocytes both increase with age (24,25), suggesting an age-associated response to normal wear and tear. Microglial overexpression of IL-1 and astrocytic overexpression of S100$\beta$ may also be found in the brains of patients with acute head injury (26, unpublished results), with HIV infection (27), and with chronic intractable epilepsy (28,29). Of particular interest is the fact that normal aging and head trauma are both recognized risk factors for the development of Alzheimer's disease (30,31), suggesting that the activation of cytokine-producing glia in these conditions may underlie this elevated risk. Neither HIV infection nor chronic epilepsy are established risk factors for Alzheimer's disease, but patients with HIV infection have limited life expectancies, and patients with epilepsy do show accelerated Alzheimer-like 'senile' changes (32).

## ROLE OF GLIAL CYTOKINES IN DISEASE PROPAGATION

In Alzheimer's disease, the activation of microglia and astrocytes and the elaboration of microglial and astrocytic cytokines proceed far beyond the modest levels associated with normal aging (24,25) or with other forms of limited neural injury (28,29). The inevitably progressive nature of Alzheimer's disease suggests that some threshold has been crossed, and that the entire process is now self-sustaining. Our elucidation of the patterns of activated glial involvement in plaque progression in Alzheimer's disease, together with the known interactions of activated glia, neurons, glial cytokines, and other molecules important in Alzheimer's disease, allow for a mechanistic explanation of the progressive, self-sustaining nature of this disease. Self propagation may occur as a result of neuronal and neuritic injury induced either directly or indirectly by the excessive glial activation and glial cytokine production within neuritic plaques. For instance, astrocytic overexpression of S100$\beta$ may tax neurons through demands for neurite outgrowth (23), compromise neuronal integrity through promotion of elevated intracellular free calcium levels (33), and stimulate astrocytic production of the potentially neurotoxic molecule nitric oxide (34). IL-1-induced neuritic overexpression of $\beta$-APP may lead to release of $\beta$-amyloid. $\beta$-amyloid may itself be neurotoxic (35). In addition, $\beta$-amyloid activates the classical complement pathway (10), an effect that may be enhanced by increased intra-plaque concentrations of the C3 complement protein (10), possibly resulting from IL-1-induced stimulation of astrocytic C3 synthesis (20). All of these potentially neurotoxic effects of glial activation and cytokine overexpression (direct neuronal overstimulation, increased nitric oxide production, and $\beta$-amyloid and complement-mediated neurotoxicity) themselves further activate microglia and increase microglial IL-1 overexpression. The entire process thus genertes a cycle, called the *cytokine cycle* (36) that, beyond some threshold of activity, can sustain itself. In this cytokine cycle, microglial IL-1 overexpression *i)* promotes astrocyte activation and upregulates astrocytic expression of S100$\beta$, ApoE, $\alpha_1$-antichymotrypsin and the complement protein C3; *ii*) stimulates neuronal synthesis and processing of $\beta$-APP; and *iii)* has autocrine effects to activate microglia and to further promote IL-1 expression. Astrocytic S100$\beta$, in turn, *i*) increases intracellular free calcium concentrations, *ii)* promotes growth of neuronal processes that, coincidentally, necessitate further neuronal expression of $\beta$-APR favoring release of neurotoxic $\beta$-amyloid; and *iii)* induces astrocytic nitric oxide synthase activity with release of potentially neurotoxic nitric oxide. The resultant neuronal cell dysfunction and death, together with $\beta$-amyloid activation of the classical complement pathway, trigger further microglial activation and IL-1 overexpression, thus turning the cytokine cycle and driving progression of both the neuropathological manifestations and clinical course of Alzheimer's disease.

## ROLE OF ACTIVATED GLIA AND OF GLIAL CYTOKINES IN ALZHEIMER PATHOGENESIS

These proposed mechanisms of disease progression may be evaluated through studies of patients with elevated risk of later development of Alzheimer's disease. Established risk factors for late-onset, sporadic Alzheimer's disease include aging (31), the $\varepsilon$4 allele of apolipotrotein E (37), and head trauma (30). In addition, patients with Down syndrome have a nearly 100% incidence of Alzheimer-type neurodegenerative changes in their fourth and fifth decades (38), establishing Down's syndrome as a causative factor for later development of Alzheimer's disease. Systematic studies of glial activation and overexpression of glial cytokines in these predisposing conditions have confirmed the hypothesized links between IL-1 and S100$\beta$ over-expression and Alzheimer's disease. All of these conditions, either alone or in combination, have now been shown to manifest overexpression of both microglial IL-1 and astrocytic S100$\beta$. In normal aging, these glial cytokines increase

progressively, although they never reach the excessive levels found in Alzheimer's disease (24,25). Head-injured patients show a diffuse overexpression of both glial cytokines (26, unpublished data), sometimes in association with small collections of swollen β-APP-containing neurites, forming rudimentary neuritic plaque-like structures (26). Moreover, diffuse cerebral deposition of β-amyloid peptide is seen in about a third of such patients (39), particularly those head-injured patients that carry the apolipoprotein E ε4 allele (40). Down syndrome patients show excessive microglial IL-1 expression and astrocytic S100β expression even at fetal and neonatal stages of development (2), decades before the appearance of neuritic plaques or neurofibrillary tangles (38). These increases in the expression of these two cytokines might be viewed as a pathological acceleration of their normal age-related increases in expression. This, together with the proposed role of these cytokines in Alzheimer's disease progression, suggests that early overexpression of these cytokines in Down's syndrome may explain in part the early susceptibility of Down's patients to Alzheimer-type neuropathological changes. S100β is encoded by a gene on chromosome 21 (41), perhaps explaining some of the S100β overexpression in Down's syndrome, but the observed increases exceed the 1.5-fold amplification expected from gene dosage increase alone (2). In contrast, early IL-1 overexpression in Down's syndrome is not attributable to gene triplication as IL-1 is a chromosome 2 gene product (42). IL-1 overexpression in Down's syndrome must therefore be an indirect effect of trisomy 21, involving some other, unidentified, intermediate gene product.

## THERAUPETIC IMPLICATIONS

The results reviewed here suggest that the cellular and molecular forces driving plaque and disease progression in Alzheimer's disease are fundamentally immunological in nature. This suggests that novel therapeutic protocols directed toward interrupting this immunological component might be of benefit. A number of recent clinical and epidemiological observations support this idea (43–46). The first example, a retrospective analysis of pharmaceutical use by twins discordant for Alzheimer's disease, showed greater use of nonsteroidal anti-inflammatory agents in those twins with later onset and milder course of the disease (43).

## CONCLUSION

Established functions of the glia-derived cytokines discussed here suggest that plaque-associated activated glia, and the cytokines they elaborate, are integral elements of neuritic plaque pathophysiology in Alzheimer's disease. The presence of activated microglia overexpressing IL-1 and activated astrocytes overexpressing S100β in early pre-neuritic plaque lesions (diffuse non-neuritic plaques), and the characteristic waxing and waning of these activated glia with the postulated stages of neuritic plaque evolution, suggest that these glia and their cytokines are driving this progression. This suggestion is supported by the known biological activities of IL-1 and S100β. Evaluation of brains from patients dying with conditions known to predispose to Alzheimer's disease has further suggested that microglial IL-1 and astrocytic S100β overexpression are early events in Alzheimer pathogenesis. We conclude, therefore, that activated glia and glial cytokines are important initiating factors for Alzheimer's disease.

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## References


1. Dolman, CL. Textbook of Neuropathology. Davis, RL.; Robertson, DM., editors. Williams and Wilkins; Baltimore, MD: 1985. p. 117-137.
2. Griffin WST, Stanley LC, Ling C. Proc Natl Acad Sci USA. 1989; 86:7611–7615. [PubMed: 2529544]
3. Mandybur TI, Chuirazzi BA. Neurology. 1990; 40:635–639. [PubMed: 1690867]
4. Sheng JG, Mrak RE, Griffin WST. J Neurosci Res. 1994; 39:398–403. [PubMed: 7884819]
5. Das S, Potter H. Neuron. 1995; 14:447–456. [PubMed: 7857652]
6. Rus HG, Kim LM, Niculescu FI, Shin ML. J Immunol. 1992; 148:928–933. [PubMed: 1530957]
7. Abraham CR, Selkoe DJ, Potter H. Cell. 1988; 52:487–501. [PubMed: 3257719]
8. McGeer PL, Akiyama H, Itagaki S, McGeer EG. Neurosci Lett. 1989; 107:341–346. [PubMed: 2559373]
9. Sheng JG, Mrak RE, Griffin WST. Neuropathol Appl Neurobiol. 1996; 22:334–341. [PubMed: 8875468]
10. Rogers J, Cooper NR, Webster S, Schultz J, McGeer PL, Styren SD, Civin WH, Brachova L, Bradt B, Ward P. Proc Natl Acad Sci USA. 1992; 89:10016–10020. [PubMed: 1438191]
11. Griffin WST, Sheng JG, Roberts GW, Mrak RE. J Neuropathol Exp Neurol. 1995; 54:276–281. [PubMed: 7876895]
12. Crystal H, Dickson D, Fuld P, Masur D, Scott R, Mehler M, Masdeu J, Kawas C, Aronson M, Wolfson L. Neurology. 1988; 38:1682–1687. [PubMed: 3185902]
13. Katzman R, Terry R, De Teresa R, Brown T, Davies P, Fuld P, Renbing X, Peck A. Ann Neurol. 1988; 23:138–144. [PubMed: 2897823]
14. Mrak RE, Sheng JG, Griffin WST. J Neuropathol Exp Neurol. 1996; 55:273–279. [PubMed: 8786385]
15. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL. J Neuropathol Exp Neurol. 1989; 48:674–691. [PubMed: 2677252]
16. Goldgaber D, Harris HW, Hla T, Maciag T, Donnelly RG, Jacobsen JS, Vitek MP, Gajdusek DC. Proc Natl Sci USA. 1989; 86:7606–7610.
17. Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE, Greengard P. Proc Natl Acad Sci USA. 1992; 89:10075–10078. [PubMed: 1359534]
18. Barger SW, Smith-Swintosky VL, Rydel RE, Mattson M. Ann NY Acad Sci. 1993; 695:158–164. [PubMed: 8239276]
19. Giulian D, Woodward J, Young DG, Krebs JF, Lachman LB. J Neurosci. 1988; 8:2485–2490. [PubMed: 2470873]
20. Barnum SR, Jones JL. Neurosci Lett. 1995; 197:121–124. [PubMed: 8552275]
21. Sheng JG, Ito K, Skinner RD, Mrak RE, Rovnaghi CR, Van Eldik LJ, Griffin WST. Neurobiol Aging. 1996; 17:761–766. [PubMed: 8892349]
22. Marshak DR, Pesce SA, Stanley LC, Griffin WST. Neurobiol Aging. 1992; 13:1–7. [PubMed: 1371849]
23. Kligman D, Marshak DR. Proc Natl Acad Sci USA. 1985; 82:7136–7139. [PubMed: 3863143]
24. Sheng JG, Mrak RE, Rovnaghi CR, Kozlowska E, Van Eldik LJ, Griffin WST. Neurobiol Aging. 1996; 17:359–363. [PubMed: 8725896]
25. Mrak RE, Sheng JG, Griffin WST. Soc Neurosci Abstr. 1996; 22(Part 1):213.
26. Griffin WST, Sheng JG, Gentlemann SM, Graham DI, Mrak RE, Roberts GW. Neurosci Lett. 1994; 176:133–136. [PubMed: 7830934]
27. Stanley LC, Mrak RE, Woody RC, Perrot LJ, Zhang SX, Marskak DR, Nelson SJ, Griffin WST. J Neuropathol Exp Neurol. 1994; 53:231–238. [PubMed: 8176406]
28. Sheng JG, Boop FR, Mrak RE, Griffin WST. J Neurochem. 1994; 63:1872–1879. [PubMed: 7931344]
29. Griffin WST, Yeralan O, Boop F, Rovnaghi CR, Sheng JG, Mrak RE, Van Eldik LC. J Neurochem. 1995; 65:228–233. [PubMed: 7790864]

30. Gentleman S, Roberts G. Br Med J. 1992; 304:118–119. [PubMed: 1737121]
31. Tomlinson, BE. Greenfield's Neuropathology. 5. Adams, JH.; Duchen, LW.; Arnold, E., editors. Oxford Press; New York: 1992. p. 1284-1410.
32. Mackenzie IRA, Miller LA. Acta Neuropathol. 1994; 87:504–510. [PubMed: 8059603]
33. Barger SW, Van Eldik LJ. J Biol Chem. 1992; 267:9689–9694. [PubMed: 1315768]
34. Hu J, Castes R, Guevara JL, Van Eldik LJ. J Biol Chem. 1996; 271:2543–2547. [PubMed: 8576219]
35. Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE. J Neurosci. 1992; 12:376–389. [PubMed: 1346802]
36. Griffin WST, Sheng JG, Royston MC, Gentleman SM, Graham DI, Roberts GW, Mrak RE. Submitted.
37. Strittmatter WJ, Roses AD. The Neuroscientist. 1995; 1:298–306.
38. Wisniewski KE, Wisniewski HM, Wen GY. Ann Neurol. 1985; 17:278–282. [PubMed: 3158266]
39. Roberts GW, Gentleman SM, Lynch A, Graham DI. Lancet. 1991; 338:1422–1423. [PubMed: 1683421]
40. Nicoll JAR, Roberts GW, Graham DI. Nature Med. 1995; 1:135–137. [PubMed: 7585009]
41. Allore R, O'Hanlon D, Price R, Neilson K, Willard HF, Cox DR, Marks A, Dunn RJ. Science. 1988; 239:1311–1313. [PubMed: 2964086]
42. Boultwood J, Brecklon G, Birch D, Cox R. Genomics. 1989; 5:481–485. [PubMed: 2613234]
43. Breitner JC, Gau BA, Welsh KA, Plassman BL, McDonald WM, Helms MJ, Anthony JC. Neurology. 1994; 44:227–232. [PubMed: 8309563]
44. Breitner JCS, Welsh KA, Helms MJ, Gaskell PC, Gau BA, Roses AD. Neurobiol Aging. 1995; 16:523–530. [PubMed: 8544901]
45. Rich JB, Rasmusson DX, Folstein MF, Carson KA, Kawas C, Brandt J. Neurology. 1995; 45:51–55. [PubMed: 7824134]
46. Andersen K, Launer LJ, Ott A, Hoes AW, Breteler MM, Hofman A. Neurology. 1995; 45:1441–1445. [PubMed: 7644037]