Acta Neuropathol (1997) 94 : 1–5

EXPRESS COMMUNICATION

Jin G. Sheng · Robert E. Mrak · W. Sue T. Griffin

Neuritic plaque evolution in Alzheimer's disease is accompanied by transition of activated microglia from primed to enlarged to phagocytic forms

Received: 15 January 1997 / Accepted: 17 March 1997

Abstract Activated microglia, overexpressing the potent neuroactive cytokine interleukin-1, have been implicated as a driving force in the evolution of diffuse amyloid deposits into diagnostic neuritic plaques in Alzheimer's disease. To evaluate this role further, we used double-label immunohistochemistry to classify and quantify plaque-associated and non-plaque-associated activated interleukin-1-immunoreactive microglia in parahippocampal tissue from 11 patients with Alzheimer's disease. These activated microglia were subclassified as primed (only slightly enlarged), enlarged, or phagocytic (enlarged with heterogeneous cytoplasmic contents). We further determined the distribution of these microglial subtypes among four defined plaque types. Most (84%) primed microglia were not plaque associated, although 13% were present in diffuse non-neuritic plaques and 3% were present in diffuse neuritic plaques. In contrast, most enlarged (55%) and phagocytic (91%) microglia were plaque associated. Of plaque-associated enlarged microglia, most (71%) were found in diffuse neuritic plaques with the remainder evenly distributed between diffuse non-neuritic and dense-core neuritic plaques (15% each). Of plaque-associated phagocytic microglia, a few were present in diffuse non-neuritic plaques (5%), but most were found in diffuse neuritic plaques (62%) and dense-core neuritic plaques (33%). These findings show preferential association of primed microglia with diffuse amyloid deposits and imply that microglial transformation from primed, to enlarged, to phagocytic types occurs in concert with the evolution of amyloid plaques from diffuse amyloid deposits to the neuritic β-amyloid plaque forms in Alzheimer's disease. Microglial phagocytic activity in neuritic plaques may reflect involvement in the processing of diffuse amyloid into condensed β-amyloid, or in clearance of neuritic debris.

Key words Alzheimer's disease · β-Amyloid · Inflammation · Microglia · Neuritic plaque

J. G. Sheng · R. E. Mrak · W. S. T. Griffin
Department of Veterans' Affairs Medical Center,
Little Rock, AR 72205, USA

J. G. Sheng · W. S. T. Griffin
Department of Medicine,
University of Arkansas for Medical Sciences,
Little Rock, AR 72205, USA

R. E. Mrak
Department of Pathology,
University of Arkansas for Medical Sciences,
Little Rock, AR 72205, USA

R. E. Mrak · W. S. T. Griffin
Department of Anatomy,
University of Arkansas for Medical Sciences,
Little Rock, AR 72205, USA

J. G. Sheng
Department of Neurology, Rui-Jin Hospital,
Shanghai Second Medical University, Shanghai, 200025, China

W. S. T. Griffin (✉)
Geriatric Research, Education and Clinical Center,
John L. McClellan Memorial Veterans' Hospital,
4300 West 7th Street, Little Rock, AR 72205, USA
Tel.: 1-501-660-2069; Fax; 1-501-671-2524

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, in which diffuse non-neuritic amyloid deposits are thought to evolve into neuritic plaques characterized by β-pleated (congophilic) amyloid and by the appearance of swollen, irregular (dystrophic) neurites [16]. With further evolution, a dense plaque core of β-amyloid is formed. In this proposed progression, the disappearance of neuritic and cellular plaque components results in a persistent condensed core of β-amyloid – the dense-core non-neuritic (or "burned out") plaque.

Microglia are frequently associated with amyloid plaques in AD [1, 5, 13, 15]. Activated microglia, overexpressing interleukin-1 (IL-1), are found in virtually all neuritic β-amyloid plaques [5] and in most diffuse amyloid deposits [7] in AD. Plaque-associated microglia have been suggested as important elements in the transformation of supposedly benign diffuse amyloid deposits into

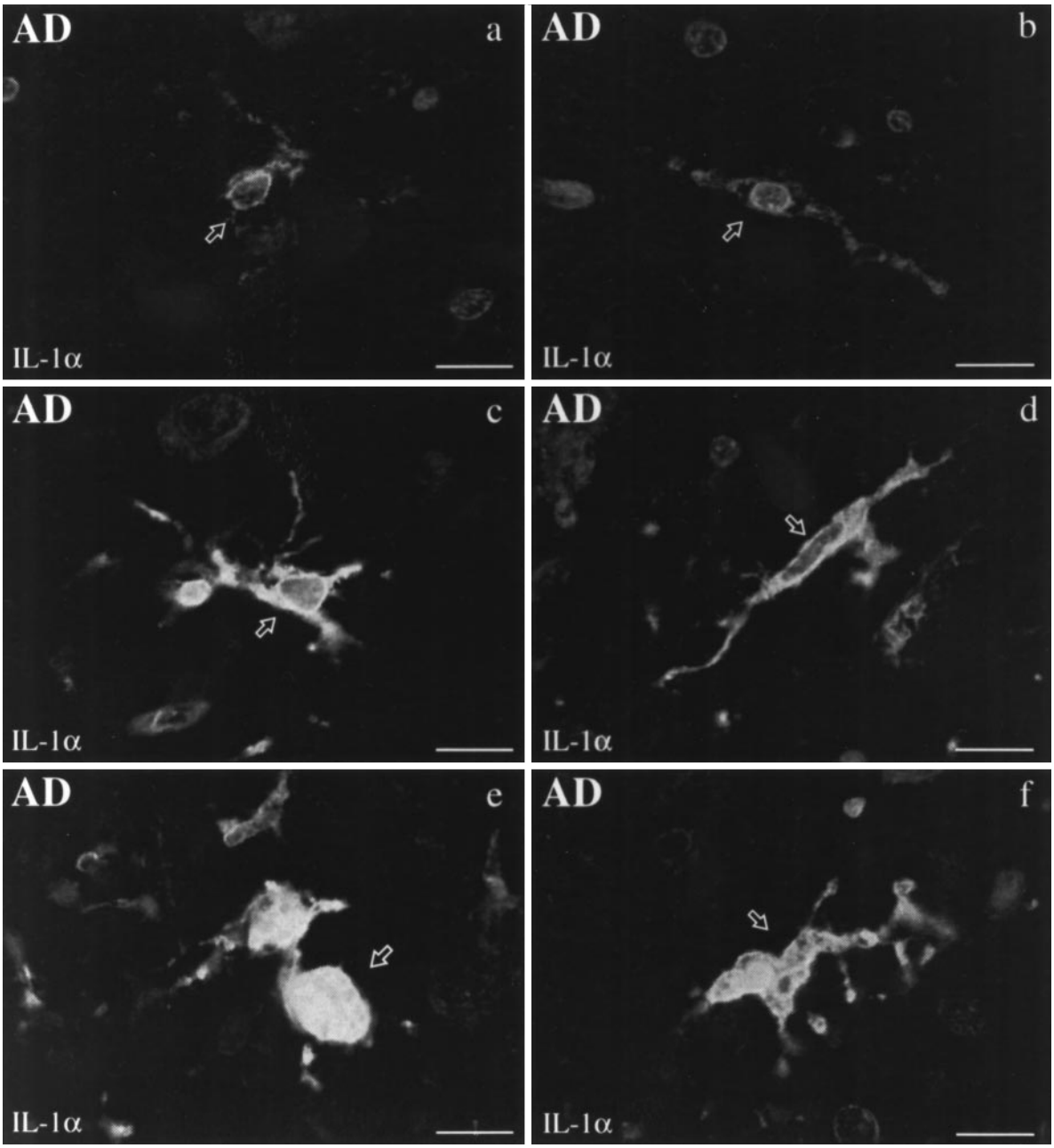

Fig. 1 a–f Photomicrographs showing activated microglial subtypes in Alzheimer's disease. Single-labelled immunohistochemistry, anti-IL-1α. **a, b** Primed microglia; **c, d** enlarged microglia; **e, f** phagocytic microglia (*IL* Interleukin, *AD* Alzheimer's disease). *Bars* **a–f** = 10 μm

the neuritic β-amyloid plaques diagnostic of AD [7, 9, 11]. The numbers of these activated microglia increase in concert with the appearance of dystrophic neurites and condensation of amyloid that occur in early stages of plaque evolution [7]. Their numbers then wane in parallel with the formation of a dense β-amyloid core [7] and with the apparent curtailment of dystrophic neurite growth in dense-core neuritic plaques [12]. Finally, microglia seem to have vanished, along with dystrophic neurites, in the end-stage, dense-core non-neuritic plaque forms. This pattern suggests a continuous involvement of microglia throughout the entire spectrum of plaque evolution. To further define this proposed involvement of activated microglia in plaque evolution, we determined the activation state of microglia and the distribution pattern of activated microglial subtypes among different plaque types in AD.

Materials and methods

Patients and tissues

Tissue was obtained postmortem from 11 demented patients (2 females and 9 males, aged 58–88 years) with neuropathological confirmation of AD according to CERAD criteria [10]. The average postmortem interval was 11 h. Right cerebral hemispheres were fixed in 20% phosphate-buffered formalin for 7–10 days prior to

coronal sectioning. For this study, tissue blocks were obtained that included hippocampus and adjacent parahippocampal gyrus, at the level of the lateral geniculate nucleus. These were then processed for paraffin embedding.

Immunohistochemistry

A polyclonal rabbit anti-human IL-1α antibody was obtained from Cistron (Pine Brook, N.H.). A monoclonal anti-human β-amyloid antibody was a gift from Dr. G.W. Roberts (SmithKline Beecham, U.K. [4]). Double and single immunolabelling was performed on 10-μm-thick paraffin sections as previously described [7].

Classification and quantification of IL-1α$^+$ microglia in different activation states

Preliminary evaluations showed that IL-1α$^+$ microglia could be classified, according to morphological and immunohistochemical criteria, as resting (small, weakly immunoreactive) or activated (variably enlarged, intensely immunoreactive). Activated IL-1α$^+$ microglia could be further classified as primed, enlarged, or phagocytic (the latter with heterogeneous cytoplasmic contents) (see Results). The numbers of each of these activated microglial subtypes were counted at × 250 in five microscopic fields (each representing 2.0 mm^2) of the parahippocampal gray matter in immunolabelled tissue sections from each of the 11 AD patients. The microglia in AD tissues were further classified as either plaque associated or non-plaque associated. Four distinct plaque types, classified as diffuse non-neuritic plaques, diffuse neuritic plaques, dense-core neuritic plaques, or dense-core non-neuritic plaques, were defined as previously described [7], and the numbers of each of the activated microglial subtypes found in association with each of these plaque types were determined.

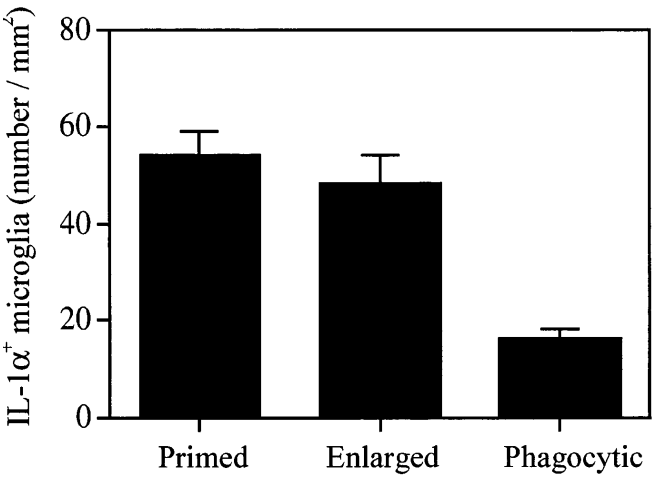

Fig. 2 Numerical density of primed, enlarged, and phagocytic IL-1α$^+$ microglia in parahippocampal cortex of AD patients. Data expressed as number of microglia/mm^2 in gray matter (mean + SEM)

Statistical analysis

Significance of differences between numbers of microglial subtypes associated with different plaque types were assessed using ANOVA followed by Fisher's test.

Results

Identification of activated microglial subtypes

Three different states of microglial activation were identified based on size and morphology: primed, enlarged, and phagocytic (illustrated in Fig. 1). Primed microglia displayed abbreviated processes and somewhat enlarged, oblong cell bodies with high nuclear-to-cytoplasmic ratios (Fig. 1 a, b). Enlarged microglia were larger and rod-shaped, with ramified processes, prominent nuclei, and abundant cytoplasm (Fig. 1 c, d). Phagocytic microglia had large cell somas, blunt short processes, and abundant cytoplasm with heterogeneous contents. The nuclei of phagocytic microglia were obscured by the heterogeneous cytoplasmic contents and the intense cytoplasmic IL-1α immunoreactivity (Fig. 1 e, f).

Quantification of IL-1α$^+$ microglia

The numbers of primed, enlarged, and phagocytic microglia in tissue sections of parahippocampal cortex from AD patients were 54 ± 5, 48 ± 3, and 16 ± 2 microglia/mm^2, respectively (Fig. 2). Many of these microglia were associated with amyloid plaques (see below). Those microglia that were not associated with amyloid plaques were widespread in areas of tangle-bearing neurons.

Quantification of plaque-associated IL-1α$^+$ microglia

The four defined plaque types (diffuse non-neuritic; diffuse neuritic; dense-core neuritic; and dense-core non-neuritic) accounted for all of the amyloid-immunoreactive plaques in the tissue sections examined. Most enlarged and phagocytic microglia were plaque-associated, while most primed microglia were not (Table 1).

Plaque-associated primed microglia were preferentially associated with diffuse non-neuritic plaques; a few primed microglia were associated with diffuse neuritic plaques. No primed microglia were found associated with either

Table 1 Immunohistochemical characteristics and distribution of activated IL-1α$^+$ microglial subtypes in the cortex of patients with Alzheimer's disease (IRI interleukin-1α immunoreactive intensity, *DnNP* diffuse non-neuritic plaque, *DNP* diffuse neuritic plaques, *DCNP* dense-core neuritic plaques, *DCnNP* dense-core non-neuritic plaques)

Subtypes	IRI	Nucleus	Distribution (% of subtype)				
			Non plaque-associated	Plaque-associated			
				DnNP	DNP	DCNP	DCnNP
Primed	++	Apparent	84%	13%	3%	0	0
Enlarged	+++	Apparent	45%	8%	39%	8%	0
Phagocytic	+++++	Obscured	9%	5%	56%	30%	0

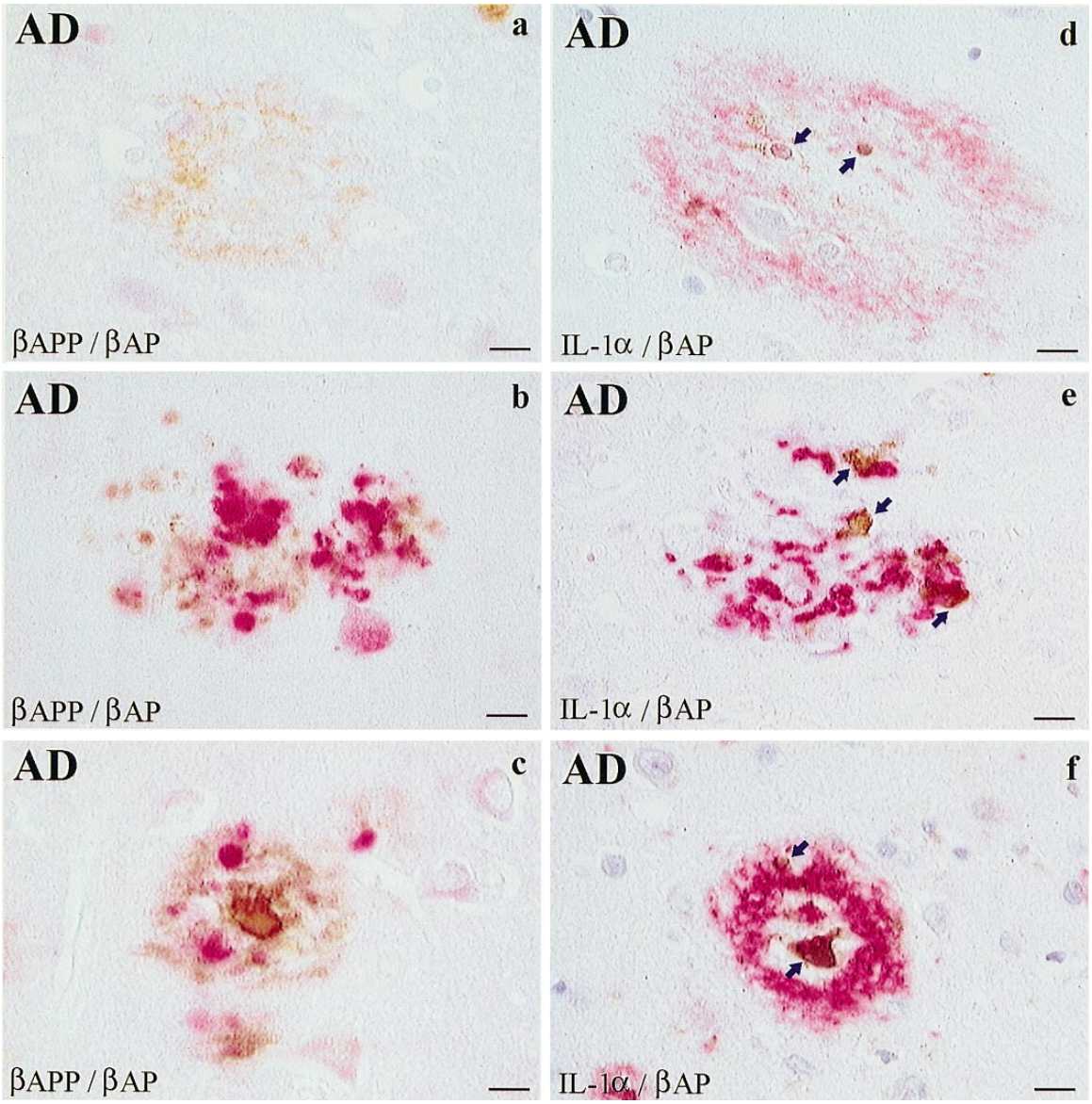

Fig. 3 a–f Examples of activated microglial subtypes associated with different plaque types in AD. The *left panels* (**a–c**) are double immunolabelled for β-amyloid precursor protein and β-amyloid *(red/brown)*. The *right panels* (**d–f**) are double immunolabelled for IL-1α and β-amyloid *(brown/red)*. **a, d** Diffuse non-neuritic plaques containing primed microglia (*arrows*, **d**). **b, e** Diffuse neuritic plaques containing enlarged microglia (*arrows*, **e**). **c, f** Dense core neuritic plaques containing enlarged and phagocytic microglia (*arrows*, **f**). *Bars* **a–f** = 10 μm

dense-core neuritic plaques or dense-core non-neuritic plaques.

Enlarged microglia were preferentially associated with diffuse neuritic plaques, while only small numbers were associated with dense-core neuritic plaques. As was the case for primed microglia, no enlarged microglia were found associated with dense-core non-neuritic plaques.

Most phagocytic microglia were found in diffuse neuritic plaques or in dense-core neuritic plaques. A few phagocytic microglia were associated with diffuse non-neuritic plaques.

Discussion

We have previously shown a distinct pattern of association between IL-1α$^+$ microglia and different plaque types, and have proposed an orchestrating role for microglia-derived IL-1 in the evolution of amyloid deposits into neuritic plaques in AD [7]. Our present results, showing that different morphological subtypes of these activated microglia are preferentially associated with different plaque types in AD, demonstrate that microglia, in addition to their role in coordinating cytokine-mediated functions, develop phagocytic activity during plaque evolution. The apparent morphological progression of plaque-associated, activated microglia across a hypothesized [7, 16] sequence of plaque evolution – from predominantly primed microglial forms in early (diffuse non-neuritic) plaque stages to predominantly enlarged forms in intermediate (diffuse neuritic) plaque stages to predominantly phagocytic forms in late (dense-core neuritic) plaque stages –

supports the idea that microglia are important pathogenic elements in plaque progression in AD.

Microglial activation, e.g., in brain injury, is characterized by enlargement, changes in shape, and increased synthesis of cytokines such as IL-1 [6]. Microglial phagocytic activity then develops in attempts to degrade potentially damaging material (for review see [19]). Our identification here of three subtypes of activated microglia – primed, enlarged, and phagocytic – suggests that overexpression of IL-1 is an early event in microglial activation, which is accompanied and followed by progressive enlargement and finally by phagocytic activity.

We find that most (84%) primed microglia (i.e., those in early stages of activation) in AD are not plaque associated. Perhaps these non-plaque-associated, cortical primed microglia are responding to the widespread neuronal damage of AD, similar to their response to neuronal injury in chronic intractable epilepsy [18] or following head injury [6]. Our observation that plaque-associated, primed microglia are most often found in diffuse non-neuritic (early stage) plaques suggests that the diffuse amyloid – and/or other amyloid-associated proteins present in early plaques – either activates resting microglia or attracts already primed microglia. Our findings that diffuse neuritic (intermediate stage) plaques contain both enlarged and phagocytic microglia, and that dense-core neuritic (late stage) plaques contain primarily phagocytic microglia suggest that morphological transformation of plaque-associated microglia occurs in concert with plaque progression, and further suggest that phagocytic microglia may contribute to the condensation of β-amyloid into dense cores during plaque evolution. This idea is consistent with previous suggestions of a microglial role in amyloid 'processing' during plaque evolution [2, 3, 8, 14]. The absence of all types of activated microglia in the vicinity of dense-core non-neuritic (end stage) plaques suggests that the fully condensed β-amyloid present in dense cores is not immunogenic. The microglia-attracting antigens in these dense β-amyloid cores may be either degraded or masked, perhaps by astrocyte-derived proteoglycans [17].

In conclusion, we find a distinct pattern of association between three types of activated microglia and four types of amyloid plaques, the latter representing stages in a hypothesized sequence of plaque evolution. These results suggest that microglial transformation – from primed to enlarged to phagocytic forms – is pathogenically related to plaque evolution, and may reflect microglial participation in the amyloid condensation that occurs during this plaque evolution. The present results, together with our previous suggestions that microglia-derived IL-1 orchestrates the astrocytic and neuritic changes that accompany this progressive amyloid condensation, implicate activated microglia as key pathogenic elements in plaque evolution.

Acknowledgements The authors wish to thank Dr. S.W. Barger for helpful suggestions on the manuscripts, Ms. S. Woodward and Ms. X.Q. Zhou for their skilled technical assistance, and Ms. P. Free for secretarial support. This research was supported in part by NIH AG10208, NIH AG12411.

References

1. Cras P, Kawai M, Siedlak S, Mulvihill P, Gambetti P, Lowery D, Gonzalez-DeWhitt P, Greenberg B, Perry G (1990) Neuronal and microglial involvement in beta-amyloid protein deposition in Alzheimer's disease. Am J Pathol 137:241–246
2. El Hachimi KH, Foncin J-F (1994) Do microglial cells phagocyte the β/A4-amyloid senile plaque core of Alzheimer disease? C R Acad Sci (Paris) 317:437–444
3. Fukumoto H, Asami-Odaka A, Suzuki N, Iwatsubo T (1996) Association of A beta 40-positive senile plaques with microglial cells in the brains of patients with Alzheimer's disease and in non-demented aged individuals. Neurodegeneration 5:13–17
4. Gentleman SM, Graham DI, Roberts GW (1993) Molecular pathology of head trauma: altered beta APP metabolism and the aetiology of Alzheimer's disease. Prog Brain Res 96:237–246
5. Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, White CL III, Araoz C (1989) Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 86:7611–7615
6. Griffin WST, Sheng JG, Gentleman SM, Mrak RE, Graham DI, Roberts GW (1994) Microglial interleukin-1α expression in human head injury: correlation with neuronal and neuritic β-amyloid precursor protein expression. Neurosci Lett 176:113–116
7. Griffin WST, Sheng JG, Roberts GW, Mrak RE (1995) Interleukin-1 expression in different plaque types in Alzheimer's disease, significance in plaque evolution. J Neuropathol Exp Neurol 54:276–281
8. Hauw JJ, Duyckaerts C, Delaere P, Chaunu MP (1988) Maladie d'alzheimer, amyliode, microglie et astrocytes. Rev Neurol (Paris) 144:155–157
9. Mackenzie IRA, Hao C, Munoz DG (1995) Role of microglia in senile plaque formation. Neurobiol Aging 16:797–804
10. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, Belle G van, Berg L (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41:479–486
11. Mrak RE, Sheng JG, Griffin WST (1995) Glial cytokines in Alzheimer's disease. Review and pathogenic implications. Hum Pathol 226:816–823
12. Mrak RE, Sheng JG, Griffin WST (1996) Correlation of astrocytic S100β expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. J Neuropathol Exp Neurol 55:273–279
13. Perlmutter LS, Barron E, Chui HC (1990) Morphological association between microglia and senile plaque amyloid in Alzheimer's disease. Neurosci Lett 119:32–36
14. Perlmutter LS, Scott SA, Barron E, Chui HC (1992) MHC class II-positive microglia in human brain: association with Alzheimer lesions. J Neurosci Res 33:549–558
15. Rozemuller JM, Eikelenboom P, Stam FC (1986) Role of microglia in plaque formation in senile dementia of the Alzheimer's type. Virchows Arch [B] 51:247–254
16. Rozemuller JM, Eikelenboom P, Stam FC, Beyreuther K, Masters CL (1989) A4 protein in Alzheimer's disease: primary and secondary cellular events in extracellular amyloid deposition. J Neuropathol Exp Neurol 48:674–691
17. Shaffer LM, Shein DS, Brannaman CA, Gambetti P, Brunden KR (1993) Processing of neuronally generated βAPP by macrophages and microglia (Abstract). J Neuropathol Exp Neurol 52:296
18. Sheng JG, Boop FA, Mrak RE, Griffin WST (1994) Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. J Neurochem 63:1872–1879
19. Streit WJ, Kincaid-Colton CA (1995) The brain immune system. Sci Am 213:54–61