Interleukin-1 Expression in Different Plaque Types in Alzheimer’s Disease: Significance in Plaque Evolution

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Abstract. The histologically apparent polymorphism of plaques containing β-amyloid in Alzheimer’s disease is thought to represent different stages in plaque evolution. β-amyloid-immunopositive plaques were classified according to the pattern of β-amyloid distribution (diffuse vs dense-core) and the presence or absence of dystrophic β-amyloid precursor protein-immunopositive (β-APP) neurites (neuritic vs non-neuritic). The potential contribution of microglia-derived interleukin-1 (IL-1), an immune response cytokine that induces synthesis and processing of β-APP, to the possible sequential development of these plaque types was examined through determination of the number of IL-1α+ microglia associated with each of four identified plaque types. Diffuse non-neuritic plaques had the least dense and most widely dispersed β-amyloid, did not exhibit β-APP+ dystrophic neurites, but most (78%) contained activated IL-1α+ microglia (2 ± 0.2/plaque; mean ± SEM). Diffuse neuritic plaques had more dense, but still widely dispersed β-amyloid, displayed a profusion of β-APP+ dystrophic neurites, and had the greatest numbers of associated activated IL-1α+ microglia (7 ± 0.8/plaque). Dense-core neuritic plaques had both compact and diffuse β-amyloid and had fewer IL-1α+ microglia (4 ± 0.4/plaque). Dense-core, non-neuritic plaques had compact β-amyloid, lacked associated diffuse β-amyloid, and were devoid of both IL-1α+ microglia and β-APP+ dystrophic neurites. These results suggest an important immunological component in the evolution of amyloid-containing plaques in Alzheimer’s disease and further suggest that IL-1-expressing cells are necessary to initiate dystrophic neurite formation in diffuse β-amyloid deposits. Our data indicate that activation of microglia with expression of IL-1 in Alzheimer’s disease is required to drive the metamorphosis of diffuse non-neuritic β-amyloid deposits to the characteristic and diagnostic neuritic plaques of Alzheimer’s disease.

Key Words: Alzheimer’s disease; Interleukin-1α; β-amyloid precursor protein; β-amyloid plaque evolution.

INTRODUCTION

β-amyloid-containing neuritic plaques are a key neuropathological feature of Alzheimer’s disease, and the number and distribution of these plaques are the basis for contemporary neuropathological diagnosis of this disease (1–3). The existence of different plaque types is well recognized, and, based on this, a progression of one plaque type to another has been suggested (4–9). The transition from more simple diffuse plaques to more complex neuritic forms is suggested by the finding that in greater than 90% of neurologically normal control patients all plaques are of the diffuse type (10), the type that predominates in younger patients with Down’s syndrome (11–13). As Rozenmuller et al (7) summarize, extracellular amorphous deposits of non-collagenic amyloid protein (termed diffuse non-neuritic plaques in our study) are thought to consolidate into collaghenic amyloid deposits that have associated dystrophic neurites, microglia, and astrocytes (diffuse neuritic plaques). This is followed by “crystallization” of the plaque amyloid into a central core (dense-core neuritic plaques) and eventually by disappearance of the neuritic and cellular elements with formation of the dense-core, non-neuritic plaques, i.e., the so-called burned-out plaques. This hypothesized sequence of plaque progression may be widely accepted, but neither the sequence of events nor the forces that drive them have been identified.

Activated glia are frequent components of plaques (14–21). These glia overexpress cytokines that have been implicated in the genesis of specific plaque features. For example, centrally located microglia in neuritic plaques (22) overexpress the potent neural and immune cytokine interleukin-1 (IL-1) (16), and plaque-associated, activated astrocyes (14, 17, 18) overexpress the neurite growth factor S100B (16, 20, 21). IL-1 is known to upregulate the expression (23, 24) and processing of β-amyloid precursor protein (β-APP) (25) and to promote the proliferation of activated astrocytes (26) and overexpression of S100B (27). The ability of both secreted S100B (20) and β-APP neurotrophic fragments (28, 29) to induce neuritic extension has been implicated, in turn, in dystrophic neurite formation in neuritic plaques (20, 21, 30). These observations suggest a pathogenic sequence in which microglia-derived IL-1 promotes the genesis of β-APP neurotrophic fragments and astrocyte-derived S100B that, in turn, promote dystrophic neurite formation in diffuse non-neuritic plaques. We examined this potential pathogenic sequence through an analysis of the distribution of

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microglia overexpressing IL-1α in four different β-amyloid-containing plaque types which represent the various stages of postulated plaque evolution.

MATERIALS AND METHODS

Patient Information and Tissue Preparation

Temporal lobe tissue sections from eight clinically demented patients (73 ± 8 years, mean ± SD), with postmortem neuropathological confirmation of Alzheimer's disease according to CERAD criteria (2, 3), were used in this study. Control tissue samples were obtained from six patients of similar age (66 ± 7 years) who had no clinical or pathological evidence of neurological disease. Postmortem intervals ranged from 2 to 26 hours (Alzheimer's disease = 10 ± 8 and control = 4 ± 1 hours). Following fixation of the left hemisphere in 20% formalin, coronal sections of hippocampus with adjacent parahippocampal gyrus and lateral geniculate nucleus were embedded in paraffin and sectioned at 10 μm.

Immunohistochemistry

For dual immunolabeling, tissue sections were pretreated with 99% formic acid for 5 minutes (min) and washed with phosphate-buffered saline (PBS). Pretreated sections were then processed as previously described (21, 31, 32) with two of the following antibodies: polyclonal anti-IL-1α (Cispron) diluted 1:20; polyclonal anti-β-amyloid (Boehringer-Mannheim Biochemical), diluted 1:10; monoclonal anti-β-amyloid (5), diluted 1:1,000; and monoclonal anti-β-APP (clone 22C11; Boehringer-Mannheim Biochemical), diluted 1:10.

Plaque Classification

Diffuse non-neuritic plaques had the least dense and most widely dispersed β-amyloid and did not exhibit β-APP+ dystrophic neurites. Diffuse neuritic plaques had more dense, but still widely dispersed β-amyloid and displayed a profusion of β-APP+ dystrophic neurites. Plaques containing round dense deposits (cores) of β-amyloid immunoreaction product as well as more diffuse β-amyloid and β-APP+ neurites were classified as dense-core neuritic plaques. Round dense deposits of β-amyloid immunoreaction product that had neither associated diffuse β-amyloid nor β-APP+ neurites were classified as dense-core, non-neuritic plaques.

Quantification of IL-1α* Cells/Plaque Type

The number of IL-1α+ cells associated with particular plaque types was counted on tissue sections dual immunoreacted for IL-1α/β-amyloid or IL-1α/β-APP. Immunoreactive structures were counted in five microscopic fields (0.4 mm²) in gray matter of parahippocampal gyrus in temporal cortex, at a magnification of 250 diameters, of an immunofluorescence tissue section from each patient. To quantitate plaque types, regions rich in plaques were chosen for counting at low magnification (100 diameters). At this low magnification, relative numbers and microglial content of different plaque types were not readily apparent on initial cursory examination, thus precluding selection bias regarding plaque type or numbers of microglial cells. Student’s t-test (unpaired data) was used to assess statistical significance of differences between numbers of IL-1α+ cells in different plaque types, representing consecutive steps in the hypothesized sequence of plaque evolution. e.g. the number of diffuse non-neuritic plaques were compared to the number in diffuse neuritic plaques and these latter were, in turn, compared to the number in dense-core neuritic plaques and so forth.

RESULTS

Figure 1 shows examples of the four different plaque types immunolabeled for β-APP, β-amyloid, and IL-1α. These four plaque types accounted for all the β-amyloid+ plaques in the sections examined. Diffuse plaques were present in the selected fields of mesial temporal lobe gray matter at a frequency of 8 ± 1 plaques/mm² (mean ± SEM) and represented 22% of all plaques. The number of diffuse neuritic plaques, those with diffuse β-amyloid+ deposits and associated dystrophic neurites overexpressing β-APP, was 18 ± 2 plaques/mm², representing 46% of all plaques. There were 5 ± 1 dense-core neuritic plaques/mm² (15% of all plaques) and 7 ± 1 dense-core, non-neuritic plaques/mm² (17% of all plaques) (Fig. 2). Therefore, of all neuritic plaques, diffuse neuritic plaques comprised 75% and dense-core neuritic plaques 25%, in close agreement with the previous findings of Masliah et al (9).

IL-1α+ microglia were apparent in many (78%), but not all of the diffuse plaques examined, with a mean of 2 ± 0.2 cells/plaque (mean ± SEM) (Fig. 3). Some additional IL-1α+ microglia were located adjacent to but not within the border of some diffuse plaques. Among the four plaque types examined, diffuse neuritic plaques had the greatest number of associated IL-1α+ microglia/plaque (7 ± 0.8). Indeed, all diffuse neuritic plaques observed contained activated IL-1α+ microglia in contrast to the observation that some diffuse non-neuritic plaques (22%) did not have IL-1α+ microglia associated with them. Dense-core neuritic plaques also almost invariably (97%) contained activated IL-1α+ microglia but there were fewer of these cells (4 ± 0.4) per plaque than in diffuse neuritic plaques. These plaques also appeared to have fewer dystrophic neurites associated with them than diffuse neuritic plaques. The IL-1α+ microglia in diffuse plaques appeared to be smaller in size (i.e. less activated) than their counterparts in both types of neuritic plaques (Fig. 1e vs 1f, g). No activated IL-1α+ microglia were observed within or adjacent to any dense-core, non-neuritic plaques in our patients (see Fig. 1h).

DISCUSSION

We found that IL-1α+ microglia were present in diffuse non-neuritic plaques, were most abundant and were larger in those plaques containing dystrophic neurites, and were not present in dense-core, non-neuritic plaques. Based on these findings and the established functions of IL-1, we suggest that the evolution of diffuse non-neuritic into diffuse neuritic plaques—a critical step in Alzheimer patho-
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 genesis (4–9, 33, 34)—is driven by the elevated levels of IL-1α derived from plaque-associated microglia. The absence of these cells in dense-core, non-neuritic plaques suggests that these plaques may represent a non-immunogenic endstage in plaque evolution.

Our finding of IL-1α+ microglia in diffuse plaques lacking neuritic components (78% of these plaques contained one or more IL-1α+ microglia) is in contrast to earlier findings (7) and has two important implications. The first is that diffuse non-neuritic plaques are not immunologically inert in cerebral cortex of patients with Alzheimer's disease. This is in contrast to the diffuse non-neuritic plaques found in the cerebellum in Alzheimer's disease (35); these do not have associated IL-1α+ microglia (36) and do not progress to neuritic forms (35, 37–39). The second implication is that microglial activation with increased IL-1α expression precedes dystrophic neurite formation and is therefore an early event in plaque evolution. This is supported by the observation that the number of activated IL-1α+ microglia was greater in diffuse neuritic plaques than in any of the other plaque types examined, which suggests a prominent role for these activated microglia and for IL-1α in the formation of dystrophic neurites, and thus in the conversion of diffuse non-neuritic plaques into the more complex neuritic plaque types. The increasing prominence of IL-1α+ microglia in diffuse non-neuritic and diffuse neuritic plaques, relative to their waning numbers in later plaque stages, supports an important role for microglia in plaque evolution, rather than a later role as suggested by Miyazono et al (40).

The observation that neither IL-1α+ microglia nor β-APP+ dystrophic neurites are present in dense-core, non-neuritic plaques suggests that IL-1 is necessary for maintenance as well as formation of dystrophic neurites. Overexpression of IL-1α may be directly related to dystrophic neurite formation via known IL-1α trophic effects on neurons (41) or by inducing processing of β-APP (25) with consequent release of neurotrophic β-APP fragments (28, 29). Alternatively, IL-1α might contribute indirectly to dystrophic neurite formation through activation of astrocytes (26) with induction of astrocyte synthesis of the neurite extension factor, S100β (27). In Alzheimer's disease, there are increased numbers of activated astrocytes containing elevated levels of biologically active S100β (20), and neuritic plaques invariably contain such astrocytes (21). Furthermore, tissue levels of S100β correlate with the density of neuritic plaques in individual Alzheimer patients (21) and with the distribution of neuritic plaques across brain regions (30).

Our results are consistent with a sequence of plaque progression (Fig. 4) wherein diffuse β-amyloid+ plaques are immunogenic, attracting and activating microglia with consequent overexpression of IL-1α. This, in turn, results in activation of astrocytes with consequent overexpression and release of S100β as well as increased expression and processing of β-APP with consequent release of neurotrophic fragments. These actions then initiate the growth of dystrophic β-APP+ neurites and thus transform diffuse non-neuritic plaques into diffuse neuritic plaques. With further condensation of β-amyloid, these diffuse neuritic plaques become dense-core neuritic plaques that are less immunogenic, thus have fewer IL-

![Fig. 1. Immunohistochemistry of plaques showing β-amyloid (β-AP) and β-amyloid precursor protein (β-APP) immunoreactive deposits and associated interleukin-1α (IL-1α) immunoreactive cells in different plaque types. Panels a–d show dual immunohistochemical staining of β-amyloid (brown) and β-APP (red): a) a diffuse non-neuritic plaque with relatively homogeneous, widely dispersed β-AP immunoreaction product and no β-APP immunoreaction product; b) a diffuse neuritic plaque with more condensed, but still widely dispersed β-amyloid and dystrophic β-APP+ neurites; c) a dense-core neuritic plaque with a compact round core deposit and a halo of less compact β-amyloid immunoreaction product and associated β-APP+ neurites; d) a dense-core, non-neuritic plaque consisting entirely of a compact, dense β-amyloid deposit. Panels e–h show dual immunohistochemical staining of β-amyloid (red) and IL-1α (brown): e) a diffuse non-neuritic plaque with two relatively small IL-1α+ microglia (arrowheads); f) a diffuse neuritic plaque with enlarged (activated) IL-1α+ microglia (arrowheads); g) a dense-core neuritic plaque with centrally located activated IL-1α+ microglia (arrowheads); h) a dense-core, non-neuritic plaque with no associated IL-1α+ cells. Bars = 15 μm.](http://jnen.oxfordjournals.org/figs/fig1.jpg)
10
# of IL-1α+ cells / plaque

Fig. 3. Number of IL-1α+ cells/plaque in four different plaque types. DnNP = diffuse non-neuritic plaques, DNP = diffuse neuritic plaques, DCNP = dense-core neuritic plaques, DChNP = dense-core, non-neuritic plaques. Data expressed as mean ± SEM for 40 plaques of each type (five in each of eight patients). For DnNP, DChNP, and DChNP, IL-1α+ cell counts were significantly different from those of the postulated predecessor plaque type (i.e. the plaque type to the left in the figure): * = p < 0.05; ** = p < 0.001.

In summary, our findings are consistent with a role for microglia overexpressing IL-1α in the evolution of plaques from simple to more complex forms in Alzheimer's disease. The fact that most diffuse plaques contained IL-1α+ microglia weighs against an alternative possibility that these microglia are reacting to rather than contributing to the induction of neurite growth. Moreover, they are consistent with our hypothesis (32, 36, 42) that the critical pathological process in Alzheimer's disease—the formation of neuritic plaques—is driven by IL-1α and the resulting molecular alchemy of acute phase responses which enable the metamorphosis of diffuse β-amyloid deposits into neuritic plaques, the harbinger of neuronal degeneration.

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Fig. 4. Proposed evolution of plaques showing involvement of IL-1α+ microglia. IL-1α+ microglia (red) in diffuse non-neuritic plaques (DnNP) (fine stippling = diffuse β-amyloid) leads to activation of astrocytes (yellow) and the appearance of dystrophic neurites (green) in diffuse neuritic plaques (DNP) (coarse stippling = condensing β-amyloid). Formation of a dense-core neuritic plaque (DCNP) (black = dense β-amyloid) is associated with diminished numbers of IL-1α+ microglia, fewer dystrophic neurites, and a lesser amount of diffuse β-amyloid. This progression may terminate in dense-core, non-neuritic plaques (DChNP), devoid of diffuse β-amyloid, IL-1α+ microglia, and dystrophic β-APP+ neurites.

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