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Glial-Neuronal Interactions in Alzheimer Disease: Progressive Association of IL-1α⁺ Microglia and S100β⁺ Astrocytes with Neurofibrillary Tangle Stages

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Abstract. Activated microglia, overexpressing interleukin-1 (IL-1), and activated astrocytes, overexpressing S100β, have been implicated in the formation and evolution of tau2-immunoreactive (tau2⁺) neuritic plaques in Alzheimer disease. In this study, we assessed the role of IL-1α⁺ microglia and S100β⁺ astrocytes in the pathogenesis of another cardinal histopathological feature of Alzheimer disease: tau2⁺ neurofibrillary tangles. Four distinct stages of neurofibrillary tangle formation were identified: neurons with granular perikaryal tau2 immunoreactivity (stage 0); fibrillar neuronal inclusions (stage 1); dense, neuronal soma-filling inclusions (stage 2); and acellular, fibrillar deposits (stage 3, "ghost tangles"). The numbers of tangles in randomly selected fields of parahippocampal cortex from 11 Alzheimer patients correlated with both the numbers of IL-1α⁺ microglia and the numbers of S100β⁺ astrocytes in these fields ($r = 0.72$, $p < 0.02$; $r = 0.73$, $p = 0.01$, respectively). There were progressive increases in frequency of association between tangle stages and both IL-1α⁺ microglia and S100β⁺ astrocytes; 48, 56, 67, and 92% of stage 0–3 tangles, respectively, had associated IL-1α⁺ microglia; and 21, 37, 55, and 91% of stage 0–3 tangles had associated S100β⁺ astrocytes. This progressive association of activated IL-1α⁺ microglia and activated S100β⁺ astrocytes with tau2⁺ tangle stages suggests a role for glial-neuronal interactions in the degeneration of tangle-bearing neurons in Alzheimer disease.

Key Words: Alzheimer disease; Astrocytes; Inflammation; Interleukin-1; Microglia; Neurofibrillary tangles; S100β.

INTRODUCTION

Neurofibrillary tangles (tangles) are hallmarks of Alzheimer disease (1, 2), and the numbers of tangles in histological sections correlate strongly with the degree of dementia in Alzheimer patients (3). These histopathological markers of neuronal degeneration and death may be found within damaged and dying neurons, or lying free in the neuropil at sites of neuronal loss (4). Ultrastructurally, tangles consist of aggregated paired helical filaments (5) that have been shown to contain high levels of an abnormally phosphorylated, microtubule-associated tau protein (6).

Neurofibrillary tangle-containing neurons show variations in tangle morphology, and this variation has been used to subclassify tangle-bearing neurons (7). Such classification has suggested a progression, from early stages characterized by abnormal delicate fibrillary argentophilic inclusions within affected nerve cell bodies to late stages in which thick, flame-shaped tangles (so-called "ghost tangles") lie free of neuronal somas in the neuropil. Intermediate between these two extremes are the classic intraneuronal tangles, characterized by highly argentophilic aggregates of fibers forming large bundles that ultimately fill the entire neuronal soma and extend into neuronal processes.

We have previously shown that amyloid plaques in Alzheimer disease contain a glial inflammatory component, characterized by the presence of IL-1-immunoreactive (IL-1⁺) activated microglia, S100β-immunoreactive (S100β⁺) activated astrocytes, and tau-immunoreactive (tau2⁺) neuronal processes (neurites) (8–12). We have proposed that overexpression of IL-1 and S100β, through established interactions that promote neurite growth, contribute to the transformation of diffuse amyloid deposits into neuritic β-amyloid plaques (13). This study evaluates the possibility that glial activation with overexpression of IL-1 and S100β might also be associated with neuronal degeneration and tangle progression in Alzheimer disease. For this, dual immunohistochemical labeling and a computerized image analysis system were utilized to quantify the association of IL-1α⁺ microglia and S100β⁺ astrocytes with tau2⁺ tangles representing different histopathological classes—and presumed different stages of evolution—of tangle formation and evolution in Alzheimer disease. Microglia overexpressing IL-1 and astrocytes overexpressing S100β show distinctive patterns of association with these different stages of tau2⁺ neurons, suggesting a pathogenic role for these cytokine-elaborating glia in tangle evolution and the accompanying neuronal degeneration.

MATERIALS AND METHODS

Patients

Eleven clinically demented patients, ranging in age from 58 to 88 years, with postmortem neuropathological confirmation of Alzheimer disease according to CERAD criteria (14), were used

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in this study. The average postmortem interval was 10 hours (h).

Antibodies

A polyclonal rabbit anti-human IL-1α was obtained from Cistron (Pine Brook, NH), and a monoclonal anti-tau antibody, tau-2, was a gift from Dr L. I. Binder (Northwestern University, Chicago, IL). This antibody is commercially available from Sigma (St., Louis, MO). Polyclonal rabbit anti-cow-S100β was a gift from Dr Linda Van Eldik (Northwestern University, Chicago, IL), but is now available from East Acres Biologicals, Inc. (Southbridge, MA) and SWant Antibodies (Bellinzona, Switzerland). The link antibodies for this study were anti-mouse or anti-rabbit IgG (Cappel, Westchester, PA).

Immunohistochemistry

Immunolabeling was performed on 10-μm-thick sections cut from 20% formalin-fixed, paraffin-embedded blocks of hippocampus and adjacent mesial temporal cortex at the level of lateral geniculate nucleus, collected postmortem and processed as previously described (15).

Single Labeling: Briefly, primary antibodies—anti-IL-1α (1:20), tau2 (1:300), or anti-S100β (1:2,000)—were diluted in 2% normal goat serum in Tris-buffered saline and were incubated on the sections overnight at room temperature.

Dual Labeling: Dual labeling kits were purchased from DAKO (Carpinteria, CA). Deparaffinized tissue sections were processed according to the manufacturer's protocol and as previously described (11). Primary antibodies employed were anti-S100β (1:2,000) plus tau2 (1:300), or anti-IL-1α (1:20) plus tau2 (1:300); all were diluted in 2% normal goat serum in Tris-buffered saline. These were applied directly to the tissue sections for incubation overnight at room temperature.

Quantification of Immunohistochemical Findings

Tangle Classification: Using the monoclonal antibody tau2, we identified four different types of neurofibrillary tangles—corresponding to those originally described by Alzheimer (4)—in formic acid-treated, formalin-fixed, paraffin-embedded tissue sections. This antibody recognizes tau protein in a phosphatase-independent manner, binding to either phosphorylated or non-phosphorylated forms of the tau protein, and is known to react immunohistochemically with neurofibrillary tangles as well as with neuropil threads, both of which contain paired helical filaments (16, 17). Tau2$^+$ (tangle-containing) neurons and cell-free tau2$^+$ tangles (ghost tangles) were classified into 4 different types according to the extent and pattern of tau immunoreactivity, as shown in Figure 1. Stage 0 tangles showed sparse, granular cytoplasmic immunoreactivity, without fibrillary structure, within otherwise morphologically normal pyramidal neuronal somas. Stage 1 tangles showed delicate, fibrillar- or rod-shaped tau2$^+$ inclusions within neuronal somas. These structures on adjacent sections were shown to be argyrophilic following treatment with the Sevier-Munger modification of Bielschowsky's stain. Stage 2 tangles appeared as classic, large, globose or flame-shaped tau2$^+$ inclusions within neuronal somas. These inclusions sometimes filled the entire neuronal cytoplasm, with displacement of the (often pyknotic) nucleus. Stage 3 tangles (ghost tangles) appeared as large, acellular bundles of loosely arranged filaments located free in the neuropil.

Counting of Immunolabeled Cells: Quantitative estimation of immunoreactive structures was performed in tangle-rich areas. The relative numbers of tangles at each stage were assessed using tau2 single-immunolabeled tissue sections, while quantification of the number of IL-1α$^+$ microglia or S100β$^+$ astrocytes associated with particular types of tau2$^+$ tangles was performed using appropriate dual-labeled sections. Association was defined as within 20 mm of the tangle-bearing neuron or ghost tangle. Counts were performed in five 250× fields, each representing 0.4 mm^2, for each patient. At this magnification there were approximately 30 neurofibrillary tangles per field.

Computerized Measurements of Number and Area of Immunoreactive Structures: Computerized analyses of the number and total immunoreactive area of tau2$^+$ neurons, IL-1α$^+$ microglia, and S100β$^+$ astrocytes were performed using a Macintosh computer coupled to a CCD video camera. Analyses were performed on 5 randomly selected rectangular ×250 fields of layers II to V in the parahippocampal cortex of appropriately immunolabeled sections from each patient. These fields were captured from corresponding positions in adjacent tissue sections. Image analysis was performed using the NIH Image program, version 1.59.

Statistical Analysis

Statistical analysis of differences between glial and neuronal cell numbers and sizes relative to tangle stages were performed using one-way ANOVA, followed by Fisher's post-hoc test.

RESULTS

Photomicrographs of the 4 types of tau2$^+$ neurofibrillary tangles, in association with IL-1α$^+$ microglia and S100β$^+$ astrocytes, are shown in Figure 1. The classic, large stage 2 tangles were the most frequent (40 ± 3/mm^2; mean ± S.E.M.), representing 52% of all tangles. Stages 0, 1 and 3 were similar in frequency (11 ± 1, 15 ± 1, and 11 ± 1/mm^2, respectively), and represented 14%, 20%, and 14%, respectively, of all tangles. Counts of IL-1α$^+$ microglia and S100β$^+$ astrocytes in these same fields, in adjacent sections immunoreacted for either IL-1α or S100β, showed significant correlations between the numbers of tau2$^+$ tangles and the numbers of either IL-1α$^+$ microglia or S100β$^+$ astrocytes (Fig. 2: $r = 0.72$, $P = 0.02$ for tau2/IL-1α; and $r = 0.73$, $P = 0.01$ for tau2/S100β). Similarly, significant correlations were obtained between the numbers of tau2$^+$ tangles and the immunoreactive areas of either IL-1α$^+$ microglia or S100β$^+$ astrocytes in these fields by computer analysis (data not shown).

Neurofibrillary tangles of all stages were frequently associated with IL-1α$^+$ microglia and S100β$^+$ astrocytes (Fig. 1). Figure 3 shows the frequency of association between either IL-1α$^+$ microglia or S100β$^+$ astrocytes and (a) neurons without neurofibrillary tangles, (b) neurons with neurofibrillary tangles, representing each of 3 tangle stages (stages 0–2), and (c) extracellular ghost tangles,

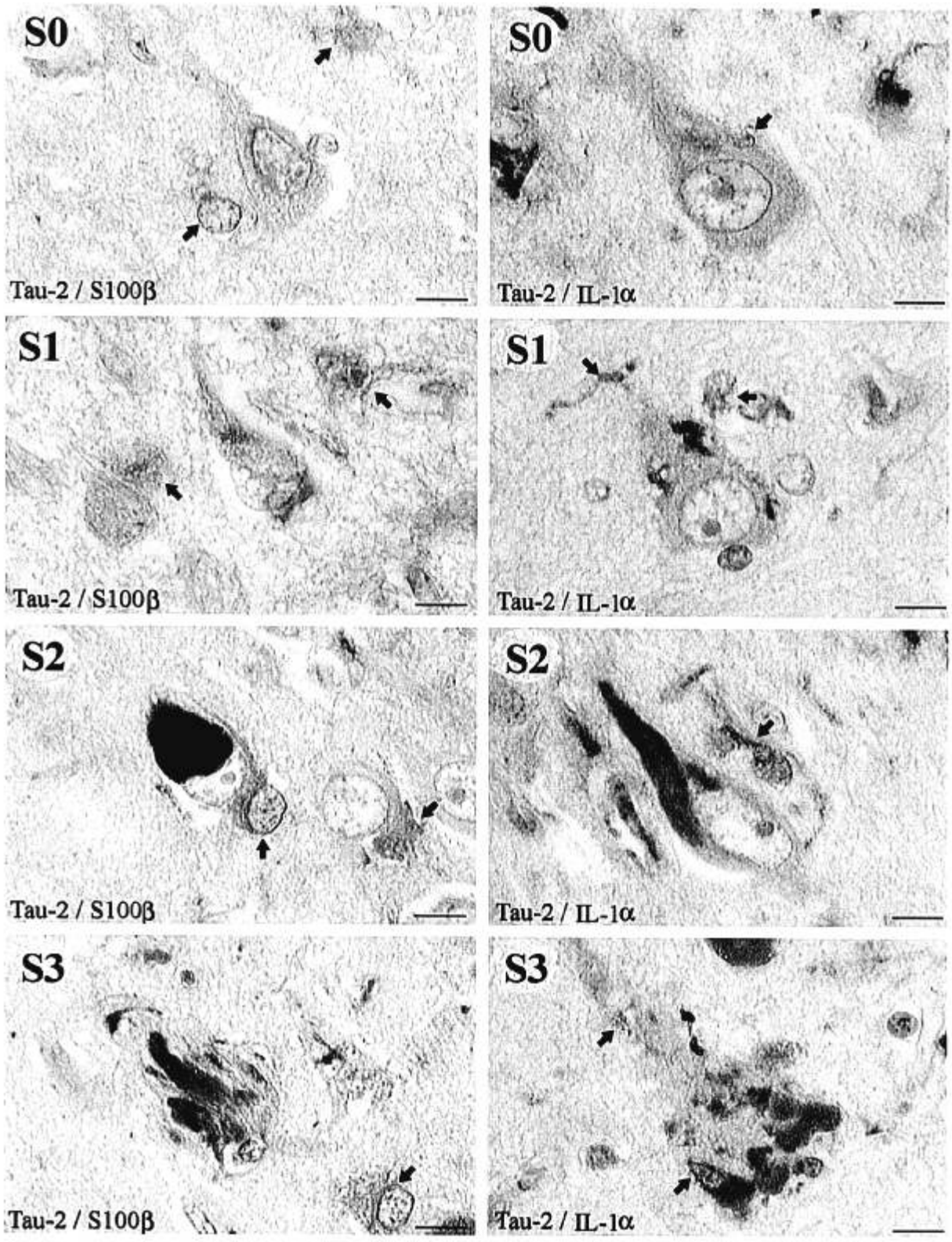

Fig. 1. Photomicrographs of hippocampal tissue sections dual-immunolabeled either for tau2 (red) and S100β (brown) (left column); or for tau2 (red) and IL-1α (brown) (right column). IL-1α$^+$ microglia (arrows) and S100β$^+$ astrocytes (arrows) are associated with tau2$^+$ tangles representing each of the 4 defined stages of tangle formation. N = normal neurons without discernible tau immunoreactivity; S0 = neurons with stage 0 tangles; S1 = neurons with stage 1 tangles; S2 = neurons with stage 2 tangles; S3 = extracellular stage 3 tangles. Bar = 10 μm.

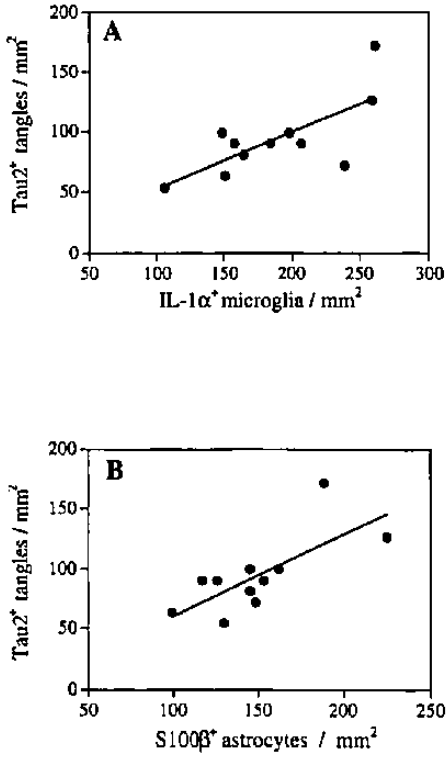

Fig. 2. Linear regression analyses of correlations between the numbers of (a) IL-1α⁺ microglia and tau2⁺ tangles; or (b) S100β⁺ astrocytes and tau2⁺ tangles, in adjacent sections of parahippocampal cortex from 11 Alzheimer patients ($r = 0.72$, $P < 0.02$ for tau2/IL-1α; and $r = 0.73$, $P < 0.02$ for tau2/S100b).

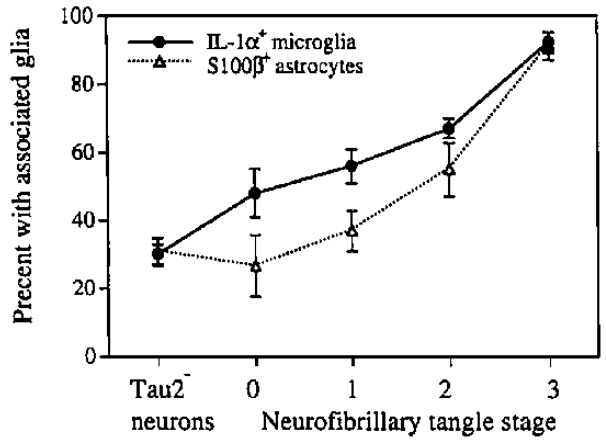

Fig. 3. Percentage of normal (tau2⁻) neurons, tau2⁺ neurons with neurofibrillary tangles at different stages (stages 0–2), and ghost tangles (stage 3) that are associated with IL-1α⁺ microglia or S100β⁺ astrocytes.

representing a fourth tangle stage (stage 3). Approximately 30% of normal (tau2 negative) neurons had associated IL-1α⁺ microglia and S100β⁺ astrocytes. IL-1α⁺ microglia were associated with nearly half of all stage 0 tangles, and this frequency of association increased steadily to reach 92% by stage 3. The frequency of association between S100β⁺ astrocytes and tangles showed a similar steady increase with stage of tangle formation, but these frequencies lagged behind those of IL-1α⁺ microglia, especially at stages 0 and 1 ($p < 0.001$). Thus, only half as many stage 0, and two-thirds as many stage 1 tangles have associated S100β⁺ astrocytes as have associated IL-1α⁺ microglia. Neither the number of IL-1α⁺ microglia nor the number of S100β⁺ astrocytes associated with neurofibrillary tangles correlated with the density of neurofibrillary tangles in the fields examined.

DISCUSSION

Our results show a significant and progressive association of both microglia overexpressing IL-1 and astrocytes overexpressing S100β with neurofibrillary tangle stages, culminating with a nearly universal (92%) association of these glia with acellular "ghost" tangles in Alzheimer disease. Intraneuronal (stages 0–2) neurofibrillary tangles were frequently associated with microglia (48–67%), in contrast to previous findings (18–20) that microglia associate almost exclusively with extracellular (stage 3) tangles (ghost tangles). These discrepancies might be accounted for by differences in microglial identification techniques (*Ricinus communis* agglutinin vs anti-IL-1α antibody) (18) or in section thickness (5–6 μm vs 10 μm) (18–20). Indeed, full-thickness sectioning of tangle-bearing neurons might reveal even higher levels of association between microglia and early tangle stages. The progressive association that we show between microglia and tangle-bearing neurons, over the entire evolutionary spectrum of tangle formation, indicates that glial-neuronal interactions precede neuronal cell death in tangle-bearing neurons, and that the microglia are not merely responding to extracellular necrotic neuronal cell remnants.

The association of microglia, overexpressing IL-1, with early stages of tangle formation suggests an elaboration of neuron-derived factors that attract and activate microglia as a result of neuronal distress (21) at the earliest stages of tangle formation. IL-1 is known to attract and activate astrocytes (22) and to induce astrocytic synthesis of S100β (23). These IL-1-based actions, together with our finding of a progressive association of S100β⁺ astrocytes with progressive stages of tau2⁺ tangle formation, suggests that these tangle-associated astrocytes were attracted and activated by microglia-derived IL-1. This idea is supported by our observation that the frequency of tangle-astrocyte association lags behind that of tangle-microglia association through the progressive stages of tangle formation.

There are potential neurotrophic and neurotoxic consequences of the attraction and activation of microglia overexpressing IL-1, and of astrocytes overexpressing

S100β, to tangle-bearing neurons. For instance, IL-1 promotes neuronal survival (24), but is neurotoxic (25) at high levels. S100β at low levels is neurotrophic, promoting neuronal survival (26) and growth of processes (27). S100β is also potentially neurotoxic through its ability to elevate intraneuronal free calcium levels (28). Furthermore, such elevation of intraneuronal free calcium concentrations may favor protein phosphorylation, including abnormal phosphorylation of tau.

We have previously shown distinctive patterns of association between amyloid plaques in Alzheimer disease and both microglia overexpressing IL-1 (9) and astrocytes overexpressing S100β (12), suggesting an important pathogenic role for these cytokines in the evolution of diffuse amyloid deposits into the neuritic plaques diagnostic of Alzheimer disease (13, 29). Our present findings, demonstrating a significant association between activated glia overexpressing these cytokines, and tangle-bearing neurons, suggest an additional pathophysiological role for glia and their cytokines in the evolution of a second cardinal histopathological feature of Alzheimer disease, the accumulation of neurofibrillary tangles. Our findings add significant weight to the concept that there is a chronic and low-grade, but pathogenically significant, glial inflammatory response that participates in the appearance and progression of 2 distinctive neuropathological features of Alzheimer disease: neuritic plaques and neurofibrillary tangles.

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