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Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease

(glia/trisomy 21/temporal lobe/neurodegenerative disorders)

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ABSTRACT **Interleukin 1, an immune response-generated cytokine that stimulates astrocyte proliferation and reactivity (astrogliosis), was present in up to 30 times as many glial cells in tissue sections of brain from patients with Down syndrome and Alzheimer disease compared with age-matched control subjects. Most interleukin 1-immunoreactive glia in Down syndrome and Alzheimer disease were classified as microglia. The number of interleukin 1 immunoreactive neurons did not appear to differ in Down syndrome and Alzheimer disease compared with control brain. Numerous temporal lobe astrocytes in Alzheimer disease and postnatal Down syndrome were intensely interleukin 1-, S-100-, and glial fibrillary acidic protein-immunoreactive and had reactive structure. Interleukin 1 levels in Alzheimer disease temporal lobe homogenates were elevated, as were the levels of S-100 and glial fibrillary acidic protein, two proteins reportedly elevated in reactive astrocytes. These data suggest that increased expression of S-100 in Down syndrome, resulting from duplication of the gene on chromosome 21 that encodes the β subunit of S-100, may be augmented by elevation of interleukin 1. As a corollary, the astrogliosis in Alzheimer disease may be promoted by elevation of interleukin 1.**

Despite the fact that Alzheimer disease (AD) and Down syndrome (DS) are distinct disorders, the neuropathology is similar in AD and in adults with DS (1). The pathophysiological changes in neurons, including the presence of paired helical filaments, as well as the extracellular accumulation of β amyloid in DS and AD temporal lobe, have been the subjects of many investigations (2). In contrast, relatively little attention has been given to the gliosis (glial proliferation and reactivity) that is a cardinal feature of AD (3–6). Gliosis has been seen in DS (7, 8) years before AD-like neuronal and extracellular changes (1).

The present study of AD and DS brain was engendered by three recent findings: (*i*) elevation of interleukin 1 (IL-1), a macrophage-derived immune response-generated cytokine (9, 10), recently demonstrated to also be produced by brain cells (11, 12), was shown to promote gliosis (13, 14), which suggested that IL-1 modulates both neural and immune events; (*ii*) the β subunit of S-100, a component of the predominant isoform of S-100 in brain (15–17), was mapped to the q22 region of chromosome 21 (18), which is duplicated in DS (19); and (*iii*) S-100 immunoreactivity was shown to be elevated in reactive protoplasmic and fibrous astrocytes (20, 21). The purpose of this study was to determine whether IL-1 expression is elevated in AD and DS temporal lobe, whether IL-1 expression is temporally related to gliosis, and whether gliosis and increased expression of S-100 in astrocytes are features of perinatal DS. Because of duplication of chromosome 21 genes, expression of S-100 at 1.5-fold normal levels would be expected in DS, but not necessarily in AD.

METHODS

Immunohistochemical techniques similar to those we have previously described (22) were used to identify IL-1-, S-100-, and glial fibrillary acidic protein (GFAP)-immunoreactive cells in 10-μm-thick sections of formalin-fixed paraffin-embedded temporal lobe from individuals with trisomy 21, a history of AD-like dementia (23), and AD neuropathology (24), as well as from individuals of similar age and postmortem interval who had neither the clinical symptoms nor the neuropathological features of AD (non-AD) and trisomy 21 (non-DS). The areas of temporal lobe examined in these studies were from similar locations in DS, AD, non-DS, and non-AD, either including or adjacent to the hippocampus.

The tissue sections were deparaffinized in toluene (three changes, 5 min each) and rehydrated in ethanol (two changes of 100% and 95%, one of 70%; 20 dips each). The cells were permeabilized with acid (0.2 M HCl, 20 min) and detergent (0.05% Triton X-100, 10 min) before endogenous peroxidase activity was blocked with H_2O_2 (0.03% in 100% methanol, 30 min) and secondary antibody/human antigen interactions were blocked with the gamma globulin (IgG) fraction of serum from nonimmune goat (Dakopatts, Glostrup, Denmark, diluted 1:5, 30 min at room temperature). All antibodies were IgG and diluted in 2% nonimmune goat serum (i.e., Tris-buffered saline 1:50, pH 7.6). The sections were then incubated overnight at room temperature with one of the following primary antibodies: (*i*) rabbit anti-human monocyte IL-1 (Genzyme, lot 08632, diluted 1:1000); (*ii*) rabbit anti-bovine brain S-100 (Dakopatts, diluted 1:300); (*iii*) rabbit anti-bovine brain GFAP (Dakopatts, diluted 1:500). As a negative control, nonimmune rabbit serum IgG (Dakopatts) was similarly incubated on adjacent sections at the same dilution as the primary antibody. A subsequent 30-min incubation with secondary antibody, goat anti-rabbit IgG (Dakopatts, diluted 1:300), was followed by a 30-min incubation with rabbit peroxidase–antiperoxidase (Dakopatts, diluted 1:50), both at room temperature. The chromogen (brown color) used to stain the immunoreactive cells was 0.044% diaminobenzidine tetrahydrochloride (Sigma)/ammonium acetate, pH 5.5/0.003% H_2O_2. Tissue sections were counterstained with Mayer's hematoxylin. Antibody specificity was tested by the manufacturer, either by absorbing out antibody activity with antigen or preabsorbing antigen with antibody.

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Abbreviations: GFAP, glial fibrillary acidic protein; AD, Alzheimer disease; DS, Down syndrome; IL-1, interleukin 1.

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IL-1-, S-100-, and GFAP-immunoreactive cells were counted in three microscopic fields at a magnification of 250 diameters in the DS, AD, non-DS, and non-AD temporal lobe tissue sections. The average cross-sectional area of five immunoreactive cells in sections from DS and non-DS brain was determined using a computer-aided reconstruction program (Biographics, Dallas). In each case, the numbers and cross-sectional areas of immunoreactive cells were determined in white matter.

Because both reactive microglia and astrocytes are present near plaques in AD (25), the distribution of IL-1-immunoreactive product within populations of astrocytes and microglia-like cells was assessed in gray matter containing plaques. In AD temporal lobe gray matter, the percentage of IL-1-immunoreactive glia that were not astrocytes (i.e., did not contain GFAP-immunoreactive product and/or were small) was estimated in adjacent sections. To obtain this percentage, the numbers of IL-1- and GFAP-immunoreactive glia were counted in three microscopic fields, at a magnification of 250×, in adjacent tissue sections immunoreacted with IL-1 antibody or GFAP antibody or with both. A total of nine fields were counted from three adjacent sections.

Western (immunologic) analysis was used to determine the steady-state levels of IL-1, S-100, and GFAP in temporal lobe, including or surrounding the hippocampus, of five AD and seven non-AD brains; these included one AD-like dementia with gliosis but not paired helical filaments and β-amyloid plaques (A1) and the three AD brains that were analyzed immunohistochemically (A3–A5). Analysis was performed using the same region of the contralateral temporal lobe to that used for the immunohistochemical procedures. As a control, tubulin steady-state levels were similarly analyzed.

Approximately 250 mg of tissue was homogenized in phosphate-buffered saline, pH 7.6, and spun for 10 min at 15,000 × *g* at 4°C. The supernatant was analyzed for protein concentration using a Bio-Rad protein assay kit 2. Twenty-five micrograms of protein for IL-1, GFAP, and tubulin analyses, or 500 μg for S-100 analysis, was loaded on each lane of a 10% SDS gel and electrophoresed for 45 min at 150 V in a Bio-Rad Mini-Protean II apparatus. Toluidine blue staining showed that the total protein profiles of each lane were similar (data not shown). Purified GFAP (ICN) or bovine serum albumin (Sigma) were electrophoresed in adjacent lanes for generation of standard curves, and Bio-Rad prestained standards were electrophoresed for molecular weight determinations.

The proteins were electrophoretically transferred (1 hr) to nitrocellulose paper in Bio-Rad Mini-Trans-Blot chambers. The transfers were immunotreated with one of the following primary antibodies diluted in Blotto (10% nonfat dry powdered milk/phosphate-buffered saline/0.001% sodium azide): IL-1 (1:2000); mouse anti-bovine S-100 (Chemicon, 1:500); GFAP (1:5000); and rabbit anti-bovine αβ tubulin (Accurate Chemicals, 1:500). After incubation with the IL-1, S-100, or tubulin primary antibodies, filters were placed in three changes of Blotto for 10 min each and then incubated for 2 hr at room temperature with secondary antibodies (ICN, diluted to 7.5 μCi per ml of phosphate-buffered saline; 1 Ci = 37 GBq) including ^{125}I-labeled goat anti-rabbit IgG (for IL-1 and tubulin) and ^{125}I-labeled goat anti-mouse IgG (for S-100). Transfers immunotreated with GFAP primary antibodies were incubated secondarily for 2 hr at room temperature with goat anti-rabbit IgG (Bio-Rad, diluted 1:2000 in Blotto) conjugated to alkaline phosphatase. Subsequently, the filters were washed again in Blotto, blotted dry with Whatman filter paper, and then exposed to Kodak XAR-5 film for 2 hr or, to visualize the immunoreactive products of GFAP, stained with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma). Immunoblots were scanned using a Beckman DU-62 spectrophotometric analysis program for comparison to a scan of a standard curve generated using purified GFAP that was similarly electrophoresed and immunotreated. Homogenates were run in triplicate from three different tissue samples from each brain.

RESULTS

The number of IL-1-immunoreactive glia per area was greater in both AD and DS than in non-AD or non-DS—as much as 30-fold in DS (Fig. 1*A*). Glial cells in postnatal DS and in AD contained intensely stained IL-1-immunoreactive product and exhibited reactive structure—i.e., enlarged somas, eccentrically placed nuclei, and prominent processes (Fig. 2 *a*, *b*, and *e*). The number of IL-1-immunoreactive neurons appeared unrelated to the attendant AD or DS. IL-1 immunoreactivity was also noted in vascular cells as reported (10). The levels of IL-1-immunoreactive product were markedly elevated in AD (A2–A5) and AD-like dementia with gliosis (A1) compared with non-AD (C1) temporal lobe homogenates (Fig. 3*a*).

The presence of IL-1-immunoreactive staining in cells was consistent with previous findings showing the production of biologically active IL-1 by astrocytes (11) and microglia (12) and the presence of IL-1 immunoreactivity in neurons and axons (30). The average cross-sectional area of IL-1-im-

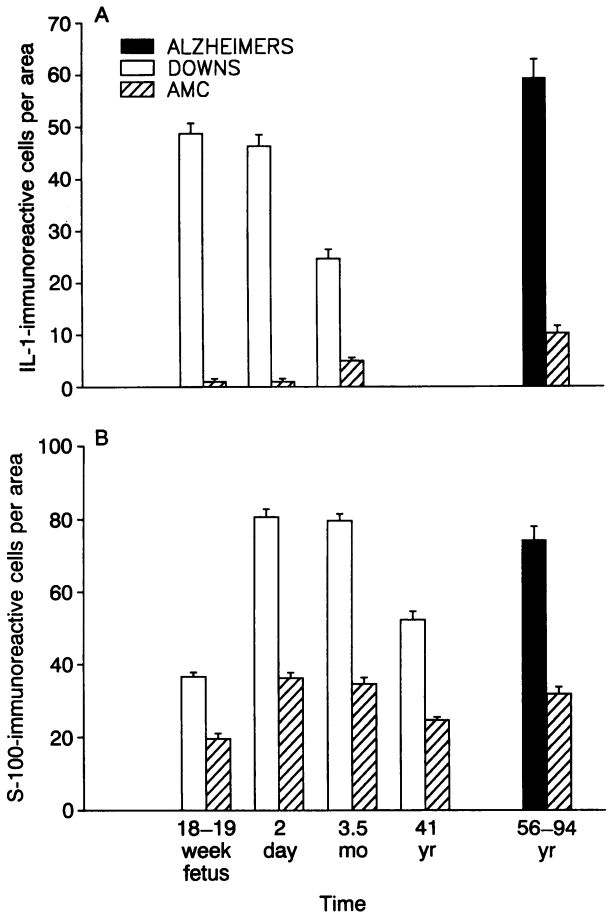

FIG. 1. Histograms depicting the mean ± SEM of the number of IL-1 (*A*) and S-100 (*B*) -immunoreactive cells in DS (karyotypically identified as trisomy 21) and non-DS (age-matched controls, AMC) fetus, neonate, infant, and adult (S-100 only) and three AD and three non-AD (AMC) temporal lobes. Cells were counted from three microscopic fields (250×) in analogous temporal lobe sections from each brain. The contralateral temporal lobe of the AD brains supplied homogenates (A3–A5) in Fig. 3.

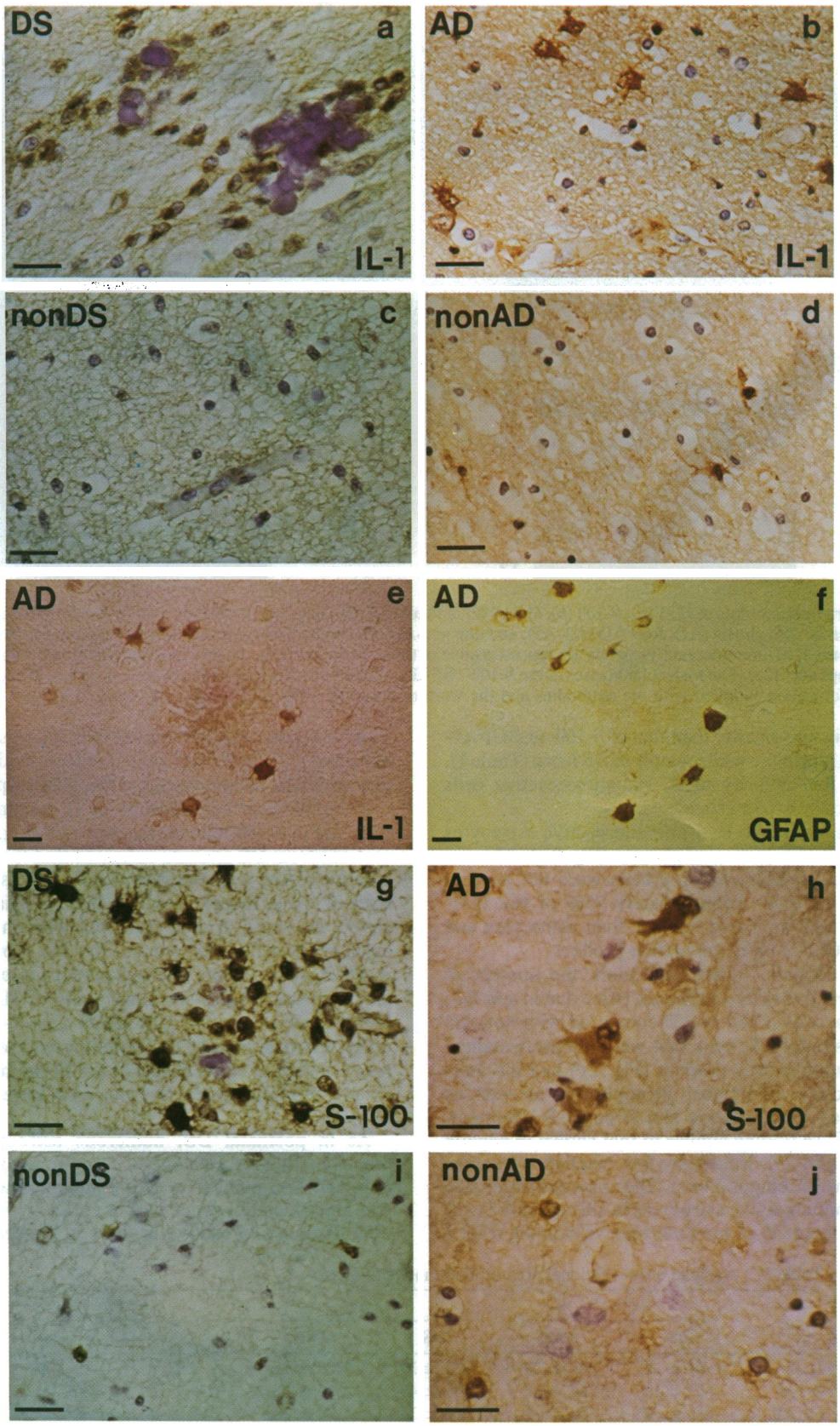

FIG. 2. Photomicrographs of IL-1-, GFAP-, and S-100-immunoreactive glia in 10-μm-thick formalin-fixed paraffin-embedded sections of temporal lobe from AD, DS, and age-matched controls (non-DS and non-AD). IL-1-immunoreactive cells (brown) tentatively identified as microglia, based on size and shape, in 2-day-old DS neonate (*a*) for comparison with age-matched non-DS (*c*). IL-1-immunoreactive glia (brown) in AD (*b*) for comparison with non-AD (*d*). Adjacent sections of IL-1 (red) (*e*) and GFAP (brown) (*f*) -immunoreactive astrocytes bordering a senile plaque in AD brain. S-100-immunoreactive astrocytes (brown) (*g*) in 2-day-old DS neonate for comparison with age-matched non-DS (*i*). Clusters of calcium deposits (purple) in DS brain may be noted in *a* and *g*. (Bar in each photomicrograph = 20 μm.)

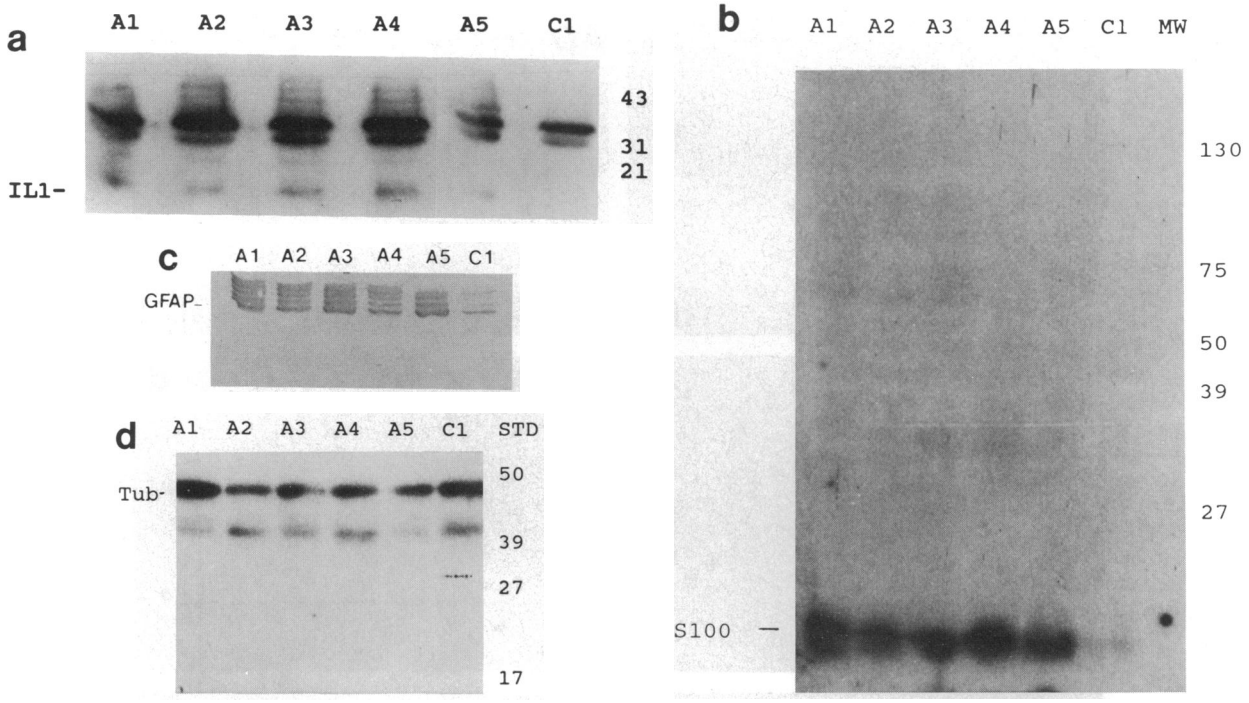

FIG. 3. Western immunoblots of IL-1 (*a*), S-100 (*b*), GFAP (c), and tubulin (*d*) -immunoreactive product in temporal lobe homogenates from one AD-like dementia with gliosis (A1), four AD (A2–A5), and one non-AD (C1) brains; six other non-AD homogenates were compared but are not shown. The three IL-1-immunoreactive bands are representative of those expected from the antibody manufacturer's data and from studies of activated macrophages (26). The molecular masses of the S-100-, GFAP-, and tubulin (Tub)-immunoreactive products are consistent with those expected from information supplied with the antibodies and the work of others (27–29). STD, molecular mass in kDa.

munoreactive glia was smaller than that of S-100- and GFAP-immunoreactive glia in the white matter of DS brain (Table 1), suggesting that the majority of IL-1-immunoreactive cells were microglia rather than astrocytes.

In gray matter from AD, IL-1-immunoreactive astrocytes were frequently clustered around plaques (Fig. 2 *e*), whereas microglia-like IL-1-immunoreactive cells were not as confined to plaque corona. Approximately 75% of the IL-1-immunoreactive glia appeared smaller than astrocytes and did not contain GFAP-immunoreactive product.

In DS fetus, neonate, infant, and adult, the number of S-100-immunoreactive cells was at least twice that in non-DS; a similar increase was noted in AD compared with non-AD (Fig. 1*B*). In postnatal DS, cells that were intensely stained with S-100 immunoreactive product (Fig. 2*g*) had the morphology of reactive astrocytes. Protoplasmic as well as fibrous astrocytes were reactive and strongly S-100-immunoreactive in DS—a finding similar to that shown in hepatic encephalopathy (20, 21). Relatively few reactive astrocytes were seen in non-DS temporal lobe. The apparent larger size of the S-100-immunoreactive astrocytes in DS compared with non-DS and the intensity of immunoreactive staining (Fig. 2 *g* and *i*) suggest that the amount of S-100 was greater than that expected (1.5-fold) from gene duplication alone and indicate that ancillary factors augment the glial responses in DS.

Morphological comparisons of IL-1-immunoreactive glia (Fig. 2*a*) to S-100-immunoreactive glia (Fig. 2*g*) on adjacent sections from DS neonate indicated that the S-100 cells were reactive astrocytes, whereas the IL-1 cells were microglia; both of these morphologically distinct glial cell types are shown clustered around elements with the appearance of calcium deposits. Our conclusion that most IL-1-immunoreactive glia in DS are microglia is supported by comparison of the average cross-sectional areas of IL-1-, S-100-, and GFAP-immunoreactive glia in DS (Table 1). The average cross-sectional area of IL-1-immunoreactive glia in DS was less than that of either S-100- or GFAP-immunoreactive glia. Overall, the immunoreactive glia in DS were larger than those in non-DS.

As in postnatal DS, numerous astrocytes in AD had reactive structure. Relatively few astrocytes were reactive in non-AD. The number of S-100-immunoreactive glia in AD was approximately twice that in non-AD (Fig. 1*B*). Amounts of S-100-immunoreactive product in the reactive astrocytes

Table 1. Average cross-sectional area of immunoreactive cells

	2 days*		3.5 mo		34 yr	
	DS	AMC	DS	AMC	DS	AMC
IL-1	84 ± 10	44 ± 10	301 ± 39	100 ± 11	ND	ND
S-100	112 ± 10	65 ± 7	550 ± 43	70 ± 8	337 ± 31	174 ± 21
GFAP	152 ± 6	80 ± 5	608 ± 35	79 ± 10	365 ± 42	114 ± 26

Average cross-sectional areas were measured in five cells per temporal lobe section by using a computer-aided reconstruction package (Biographics, Dallas). Criteria for cell selection included a white-matter location, the presence of immunoreactive product, and a visible nucleolus. Values represent the mean ± SEM of the cross-sectional area (μm^2) of immunoreactive cells. At each age for each protein, DS (trisomy 21) values are significantly greater ($P \leq 0.05$) than values for age-matched controls (AMC; non-DS). ND, not done.

*Age at postmortem examination.

in AD appeared to be elevated (Fig. 2 *h* compared with *j*), suggesting that factor(s) acting as stimulant(s) (perhaps IL-1) of astrocyte reactivity and S-100 expression are present in AD, as we suspect they are in DS. The observation of numerous reactive astrocytes filled with GFAP-immunoreactive product in AD (Fig. 2*f*) and elevated IL-1, S-100, and GFAP levels in temporal lobe homogenates of AD compared with non-AD (Fig. 3 *a–c*) support this suggestion. There was ≈3-fold more IL-1, 4-fold more S-100, and 6-fold more GFAP in AD (A2–A5) and AD-like dementia with gliosis than in non-AD (C1) temporal lobe homogenates. The steady-state levels of tubulin were not different in AD and non-AD (Fig. 3*d*).

DISCUSSION

In addition to its ability to promote gliosis (13, 14), IL-1 induces excessive expression of the β-amyloid precursor gene (31) and α_1-antichymotrypsin (32). β-Amyloid (33) and α_1-antichymotrypsin (34) both appear to be components of extracellular amyloid deposits in AD. Elevated levels of brain-derived IL-1 could, therefore, contribute to increased expression of β-amyloid precursor protein and α_1-antichymotrypsin and to the conspicuous astrogliosis in AD and DS.

Detection of elevated levels of IL-1 immunoreactivity before astrogliosis would suggest that IL-1 is involved in the regulation of astrocyte reactivity associated with increased GFAP and S-100 expression. Because of the limited number of DS cases examined, exact timing of the induction of brain-derived IL-1 in DS, relative to either the appearance of reactive astrocytes or the increase in the numbers of S-100 and GFAP-immunoreactive astrocytes, could not be firmly established in our studies. A temporal relationship was suggested by the observation of many IL-1-immunoreactive cells in the fetus, preceding the postnatal appearance of reactive astrocytes. In the DS neonate, β-amyloid immunoreactivity was not detected in brain sections adjacent to those with prominent gliosis and numerous IL-1- and S-100-immunoreactive cells. However, β-amyloid immunoreactivity has recently been reported in an adolescent with DS (35) and was observed in the plaques in our 34-year-old patient (36).

Possible regulatory interactions between IL-1 and S-100 and their roles in brain function are not yet clear. Elevation of IL-1 in DS cannot directly be assigned to gene duplication because there is no evidence that the gene(s) encoding IL-1 maps to chromosome 21 (37). If the neuropathological findings in DS are solely the result of duplication of chromosome 21 genes, the implication is that chromosome 21 gene product(s) serve as direct or indirect stimulant(s) of excessive IL-1 expression in brain cells of DS patients. By analogy, similar stimulant(s) may be activated in AD.

Our findings of increased expression of IL-1 in DS and AD and gliosis in neonatal DS suggest that changes in glia may presage neuronal and extracellular changes. This concept may not be confined to AD and DS but rather may be a generalization that applies to other diseases where gliosis is a major neuropathological finding, e.g., AD-like dementia with gliosis. We have preliminary evidence of profound gliosis with elevated IL-1 immunoreactivity in reactive glia in AIDS (W.S.T.G., L.C.S., R. C. Woody, and R. E. Mrak, unpublished data). Therefore, we suggest that although independent factors contribute to the etiology of neural diseases, those with similar neuropathologies probably involve common pathophysiological events, such as induction of IL-1 expression in brain cells and gliosis.

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