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Coexistence of reactive plasticity and neurodegeneration in Alzheimer diseased brains

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Summary. Alzheimer's disease (AD) is a pathological process characterized by neuron degeneration and, as recently suggested, brain plasticity. In this work, we compared the reactive plasticity in AD brains associated to O-glycosydically linked glycans, recognized by lectins from *Amaranthus leucocarpus* (ALL) and *Macrobrachium rosenbergii* (MRL), and the tau neuritic degeneration. The neuritic degenerative process was evaluated by the quantification of aggregated neuritic structures. Lesions were determined using antibodies against hyperphosphorylated-tau (AD2), amyloid-ß, and synaptophysin. In these conditions, we classified and quantified three pathological structures associated to the neuritic degenerative process: 1) Amyloid-ß deposits (AßDs), 2) Classic neuritic plaques (NPs), and 3) Dystrophic neurites clusters (DNCs) lacking amyloid-ß deposits. Reactive plasticity structures were constituted by meganeuritic clusters (MCs) and peri-neuronal sprouting in neurons of the CA4 region of the hippocampus, immunoreactive to synaptophysin (exclusively in AD brains) and GAP-43. Besides, MCs were associated to sialylated O-glycosydically linked glycans as determined by positive labeling with ALL and MRL. Considering that these lectins are specific for the synaptic sprouting process in AD, our results suggest the co-occurrence of of several areas of reactive plasticity and neuron degeneration in AD.

Key words: Alzheimer's disease, Amyloid-ß, Tau, Brain plasticity, ApoE

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Introduction

Alzheimer's disease is characterized pathologically by the appearance of parenchymal amyloid-ß deposits with and without neuritic elements, and by intraneuronal changes, including neurofibrillary tangles (NFTs) and synaptic loss (Geddes et al., 1985; Ihara, 1988; Terry et al., 1991). Reactive plasticity, which includes axonal and dendritic sprouting and synaptogenesis, has been proposed to contribute to the pathogenesis of several neurological disorders (Zhan et al., 1993; DeKosky et al., 1996; Espinosa et al., 2001). Neuritic plaques are formed by an amyloid-ß deposit that is surrounded by dystrophic neurites, reactive astrocytes, and microglia (Terry et al., 1964; Terry and Wisnieswski, 1972). Tau protein is the main component of NFTs and dystrophic neurites (Tomlinson et al., 1970; Wischik et al., 1985; Braak et al., 1986; Alafuzoff et al., 1987; Wischik et al., 1988; Arriagada et al., 1992). It has been suggested that in AD the number of NFTs correlates better with the severity of the dementia than with amyloid-ß deposits (Tomlinson et al., 1970; Alafuzoff et al., 1987; Arriagada et al., 1992). Normal elderly brains, as well as cognitively impaired non-demented subjects, often show many diffuse senile plaques (Delaère et al., 1990, 1993; Selkoe 1992). Similar results have been found in both retrospective (Crystal et al., 1988; Katzman et al., 1988; Tagliavini et al., 1988) and prospective studies (Delaère et al., 1991; Dickson et al., 1992). Synaptic pathology has been considered particularly relevant in several neuropsychiatric disorders. A synaptic failure may potentiate cognitive impairment and lead to deep dementia conditions (Zhan et al., 1993; DeKosky et al., 1996). Abnormally dilated synaptic terminals have been observed in both normally aging (Braak and Braak 1988) and dementia brains (Ferrer et al., 1990; Espinosa et al., 2001, 2003), these structures have been identified inside the neuritic plaques as well as in the neuropil (Espinosa

et al., 2001; 2003). Dilated synaptic terminals have been associated with aberrant regeneration in plaques or with plasticity response to degeneration, as well as with the growth-associated protein, GAP43 (Davisson and Blennow, 1998; Bogdanovic et al., 2000), but not directly related to the ApoE genotype of AD patients (Blennow et al., 1996). Recently it has also been shown that, in addition to these lesions, abnormal synaptic immunoreactivity is present in the cortex and in the hippocampal formation of AD brains. Most of the studies addressing this synaptic pathology were focused on measuring synaptic densities and, interestingly, several reports have found good correlations between synaptic density decrease and cognitive impairment. It has been proposed that synaptic loss is an early phenomenon possibly preceding loss of neurons (Masliah, 2001; Masliah et al., 2001, 2003). Synaptic sprouting has attained increasing attention as being central but it may contribute to the vulnerability to neurodegenerative disease (Teter and Ashford, 2002).

Several post-translational modifications of the tau protein have also been reported, including hyperphosphorylation (Grundke-Iqbalet al., 1986; Flament and Delacourte 1989), oxidation (Yan et al., 1994; Smith et al., 1996), glycation (Ledesma et al., 1994; Smith et al., 1994; Yan et al., 1994), and glycosylation (Wang et al., 1996; Guevara et al., 1998). Recent findings indicate that different proteins seem to participate in the major events of AD pathogenesis. These proteins, amyloid-ß precursor, tau and, recently described, the 90Azgp protein (Espinosa et al., 2003) seem to exist in several isoforms that, in many tissues, arise by alternative splicing of a single gene, but subjected to post-translational modification, such as glycosylation, particularly, O-glycosylation and sialic acid transference during transit through the trans-Golgi and the intracellular protein secretory pathway (Weidemann et al., 1989; Espinosa et al., 2003). The lectins from *Amaranthus leucocarpus* (ALL) and *Macrobrachium rosenbergii* (MRL) have been used to demonstrate post-translational modifications due to glycosylation of amyloid-ß and tau proteins in human brains from patients with Alzheimer's disease; furthermore, it has been suggested that structures recognized by these lectins seem to correlate with neuroplasticity events (Guevara et al., 1998; Espinosa et al., 2001, 2003).

The present study was aimed at identifying the coexistence of the neuritic degeneration process (evaluated by the quantification of AßDs, NPs, and DNCs) and the reactive plasticity assessed by the interaction of ALL and MRL lectins with meganeurites in AD.

Materials and methods

Tissue

Cortex tissue fragments ranging in size from 0.3 to 0.5x2x2 cm from 11 AD cases (mean age of 76.0±5.5 years) and 13 non-demented control brains (NC) without any neurological impairment (mean age of 74.1±6.9 years) were selected for this study (Table 1). AD brains presented in average more than 23 NFTs/mm^2, whereas the same areas from non-demented cases depicted in average less than 1 NFT (only in two cases). Autopsied brains were ascertained both clinically and histopathologically, according to NINCDS-ADRDA criteria (Khachaturian, 1985; Mirra et al., 1991). A neuropathological study with amyloid-ß and hyperphosphorylated-tau (monoclonal antibody AD2) was performed in four fragments of cortical associative areas, including Brodmann's areas 10-46 (prefrontal), 21-22-42 (middle/superior temporal), 39 (angular gyrus), and 40 (supramarginal gyrus). Tissue was sampled within 24 h after death and fixed by immersion in 4% paraformaldehyde for at least 36 h.

All patients included in this study were followed within the IMAGE project, a population-based study conducted in the Saguenay-Lac Saint Jean, province of Quebec, Canada (Gauvreau et al., 1988). In this project, patients underwent a full diagnostic procedure, including neurological examination, laboratory tests, and neuroimaging studies with prospective profiles and longitudinal studies for cognitive deficits (Joanette et al., 1994). By the time of death, autopsy was performed when the family gave written consent. ApoE genotype was routinely performed according to a previous report (Betard et al., 1994). Data of genotype were available only at the end of the experiments. The subjects used in this study were not selected based on their genotype.

Immunohistochemistry

Paraffin embedded blocks from AD and normal control brains were cut 6 μm thick and single labeled according to the indirect PAP method using a monoclonal antibody (mAb) against hyper-phosphorylated PHF-tau (mAb AD2, dilution 1:500) (Buee-Scherrer et al., 1996) and against Growth-Associated Protein 43 (GAP-43), (MAB347, Chemicon International Inc. Temecula, CA. Dilution 1:500). For control assays of tau immunoreactivity on neuritic structures we used several antibodies, such as anti-Neurofibrillary tangles (AB1518 Chemicon International Inc. Temecula, CA), anti-tau (A002401 Dako Co. Carpinteria, CA), and tiazin red (TR), which is a highly specific fluorescent dye for NFTs and dystrophic neurites in AD (Guevara et al., 1998). Immunohisto-chemistry against synaptophysin protein was performed on 11 sampled cortical tissue fragments (0.3 to 0.5x2x2 cm) including Brodmann's areas (BA): (1) BA 10; (2) BA 10-46; (3) BA 6; (4) BA 21-22-42; (5) BA 7; (6) BA 40; (7) BA 39; (8) BA 19; (9) BA 28-34-38; (10) BA 27-28-35-36; (11) BA 23-30-31. Slides were labeled with mAb against human synaptophysin (MAB332, Chemicon. Dilution 1:1000). Secondary isospecific antibody HRP conjugated (Jackson Immunoresearch,

Canada) was used to develop indirectly in a standard immunoperoxidase method with DAB.

Double labeling

Selected sections from AD and NC subjects from the hippocampus and cerebral cortex including the Brodmann's areas 10-46, 21-22-42, 40 and 39 (also listed above as regions 2, 4, 6 and 7, respectively, in the plots) were processed at the same time and with the same methodology. Briefly, tissue sections were incubated overnight in a humidity chamber at 4 °C with the primary antibody AD2 in PBS pH 7.4 (dilution, 1:500). Sections were then washed in PBS for 15 min and subsequently incubated for 1 h at room temperature in isotype secondary antibody FITC-conjugated (Jackson Immunoresearch, Canada). Tissue samples were then washed and incubated with the second primary antibody, i.e., a polyclonal antibody anti-amyloid-ß (Boeringher-Manheim, Germany) (dilution, 1:50) for 2.5 h at room temperature. Slides were washed and incubated for 1 h at room temperature with the second isotype antibody, lissamine-rhodamine-conjugated (Jackson Immuno-research, Canada). Finally, the samples were washed and mounted with Vectashield (Vector laboratories, Burlingame, CA). Slides were always pre-treated in 80% formic acid for 5 min to enhance amyloid-ß immunoreactivity (Guevara et al., 1998; Espinosa et al., 2001). To monitor amyloid-ß assembly, we also performed double labeling with fluorescent TR, aqueous solution at 0.0002% for 15 min (Guevara et al., 1998; Espinosa et al., 2001).

Lectin histochemistry

ALL (specific for N-acetyl-D-galactosamine) and MRL (specific for 9-0-acetyl-sialic acid) binding was indirectly recognized on paraffin embedded samples with Extravidin-FITC-conjugated (Sigma Chemical Co., St. Louis, MO). Sections were washed in PBS for 5 min; lectins diluted in PBS (50 μg/ml) were applied for 2 h at 37 °C. Sections were thoroughly rinsed with PBS before incubating for 1 h, at 37 °C in Extravidin-FITC (Sigma, dilution 1:100) followed by PBS rinsing. Prior to mounting the slides with Vectashield (Vector), samples were counterstained with fluorescent TR, aqueous solution at 0.0002% for 15 min (Guevara et al., 1998; Espinosa et al., 2001). Control experiments consisted in omitting the lectin. Specificity of lectin interaction was determined by hapten-inhibition assays: ALL was pre-incubated with 200 mM of its specific sugar. For MRL, 10 μM bovine submaxillary gland mucin was used (Vázquez et al., 1993). Lectin interaction was also determined on slides previously incubated with 0.1 U of Vibrio cholerae sialidase (Sigma) per 100 μl of PBS at 37 °C for 30 min.

Table1. Subjects used in this study.

CASE	AD VARIANT	GENDER	ONSET (age)	AGE OF DEATH (yrs)	DURATION (yrs)	APOE GENOTYPE	POSTMORTEM (hr)
1	LB	M	76	81.7	5	3-4	5.8
2	-	F	71	78	7	4-4	23
3	-	M	66	79.4	13	2-3	11.5
4	-	F	58	81.1	23	3-4	36.5
5	-	F	58	70.4	12	4-4	9.3
6	-	F	61	70.8	9	4-4	18
7	-	F	68	76	8	3-4	19
8	-	F	68	80.5	12	4-4	12.5
9	Mixed	M	74	81.8	7	4-4	6
10	LB	M	57	68.2	11	4-4	3
11	LB	M	60	68.7	8	4-4	8
Control Subjects							
12	-	M	-	76	-	2-2	14
13	-	M	-	71	-	2-2	15
14	-	M	-	85	-	2-2	1.5
15	a	F	-	76	-	3-4	23.5
16	-	M	-	67	-	3-4	10.7
17	b	M	-	75	-	2-3	21
18	-	M	-	73	-	3-4	4
19	c	M	-	80	-	3-4	23
20	-	M	-	75	-	2-4	20
21	-	M	-	78	-	3-4	20
22	-	F	-	79	-	3-4	22
23	-	F	-	63	-	3-4	4
24	-	M	-	64	-	2-4	18

a: pathological aging without dementia; b: microinfarcts; c: communicant hydrocephaly; LB: presence of Lewy Bodies.

Confocal microscopy

Double-labeled slides were viewed with a Zeiss microscope (Zeiss, Germany) equipped with epi-illumination and a dual laser confocal system (Zeiss LSM 410). Using a 40x oil-immersion objective lens, images were consecutively captured in two channels (FITC A= 492; E= 520; lissamine-rhodamine A= 570; E= 590). The images were merged using a pseudocolor display (green for FITC and red for lissamine-rhodamine). In the merged image, a yellow color was interpreted as a co-localization between two markers. Images were stored in 250 MB optical disk cartridges and photographed on Kodak T-Max 100 film and Ektachrome 100 (Kodak, Eastman Co). Color images were printed with a Fuji color video printer.

Plaque density measurements

Plaques density was evaluated for both AD and non-demented cases. To evaluate the plaque subsets in both AD and non-demented cases we screened with a 40x oil objective all the tissue available on each slide. From 4 different brain cortical regions, all gray and white matter was screened and single fields at this magnification were scanned each time we found a plaque. A two-channel consecutive image in confocal microscopy was then captured and stored as a digitized image of 512x512 pixels. After confocal analysis, on each slide we measured the total gray matter surface in order to be able to report a true density of plaques subsets, $1/mm^2$. Since there were some variations in the extent of tissue sections, subtype densities were normalized for the area tissue by measuring with a CCD camera interfaced to an image analysis system (MCID Image Analysis System, Imaging Research Inc., ON. Canada), and reported as plaques subsets per 1 mm^2. Densities of plaques were automatically computed by image analysis (IBAS, Kontron Electronik, Germany).

Density of positive synaptophysin meganeurites

Serial sections from the 11 cortical brain regions (in both hemispheres) included in this study were scanned with a 10x objective (total magnification = 100x). Reported plaque density represented the average of the three richest fields at the same magnification. Density of anti-synaptophysin immunoreactive lesions (MCs) was reported per 1 mm^2. Student's t-test, two tails, was used to compare groups.

Results

Synaptic sprouting at CA4

The immunoreactivity to synaptophysin was observed as a diffuse dot pattern distributed throughout the neuropil. This immunoreactive dot pattern was not significantly different between AD and normal control cases. Immunoreactivity to synaptophysin was observed as peri-neuronal sprouting on neurons located in the CA4 region of the hippocampus (Fig. 1A). In AD brains, this synaptic sprouting was characterized by a peri-neuronal arrangement of meganeurites on neurons with incipient NFTs (Fig. 2C). Synaptic sprouting was infrequently observed in normal non-demented control brains.

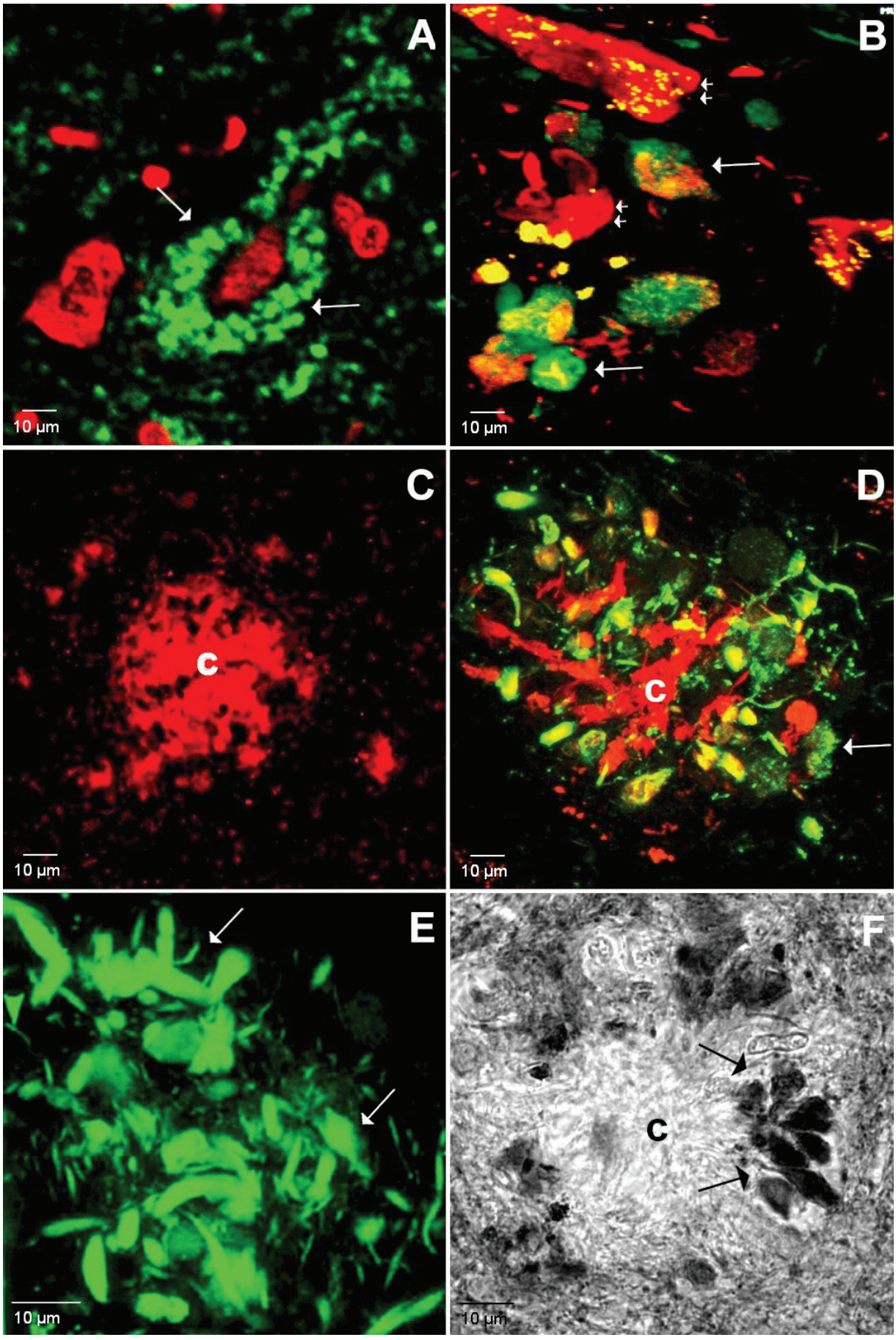
Meganeuritic sprouting in AD

ALL recognized preferentially the membrane of meganeuritic structures with a diameter bigger than 10 μm (Fig. 1B); these structures showed high tendency to form clusters, sometimes associated with amyloid-ß deposits (Fig. 1B). ALL labeled the meganeurites but not the neurofibrillary tangles recognized by TR or PHF-tau in filaments (Fig. 1B); interestingly, in these meganeurites, ALL also showed affinity for granular components. MRL labeled meganeurites and cytoplasmic and granular components. Incubation of tissue slides with *V. cholerae* sialidase rendered negative the labeling of structures by MRL (data not shown). In addition to this labeling, slides double-labeled with GAP-43 and TR revealed that synaptic sprouting was related to NFTs (Fig. 2A) and amyloid-ß deposits (Fig. 2B). Anti-GAP-43 antibody recognized meganeurites and the regular dystrophic neurites around amyloid-ß deposits (Fig. 2A) as well as neurons with incipient NFTs (Fig. 2B).

Plaque distribution

Brain sections were double-labeled to recognize simultaneously amyloid-ß protein and the hyperphosphorylated-tau protein through confocal microscopy. In our experimental conditions, anti-tau

Fig. 1. Lesions found in AD. **A.** Double labeled peri-neuronal sprouting immunoreactive to synaptophysin (arrows) rounding a neuron in CA4 region in AD brains. Confocal microscopy. **B.** Double labeled slide visualized through confocal microscopy, showing meganeurites labeled with ALL (arrows) associated to neurofibrillary tangles labeled by fluorescent tiazin red dye (arrowheads) in AD brains. **C**. Double labeled slide visualized through confocal microscopy, showing an amyloid-ß deposit (c) (red channel). This type of deposit (AßDs) is not related to dystrophic neurites (green channel). Amyloid-ß deposits were observed especially in non-demented cases, but also in AD. **D.** Double labeled NPs through confocal microscopy. Dystrophic neurites (green channel, hyperphosphorylated-tau, (arrow) around the amyloid-ß deposit or core (c) in AD brains. This is the most characteristic plaque (NPs) of AD. **E.** Double labeled slides visualized through confocal microscopy, showing dystrophic neurites in clusters (arrows). There is no amyloid-ß deposit (absence of red color). This type of neuritic cluster (DNCs) was found exclusively in AD cases. **F.** Meganeuritic clusters (MCs) immunoreactive to synaptophysin in AD cortex (arrows) and around the amyloid-ß deposit (c). The meganeurites labeled with PAP standard method are widely distributed in a radial arrangement in AD brains as determined by transmitted light channel in confocal microscopy.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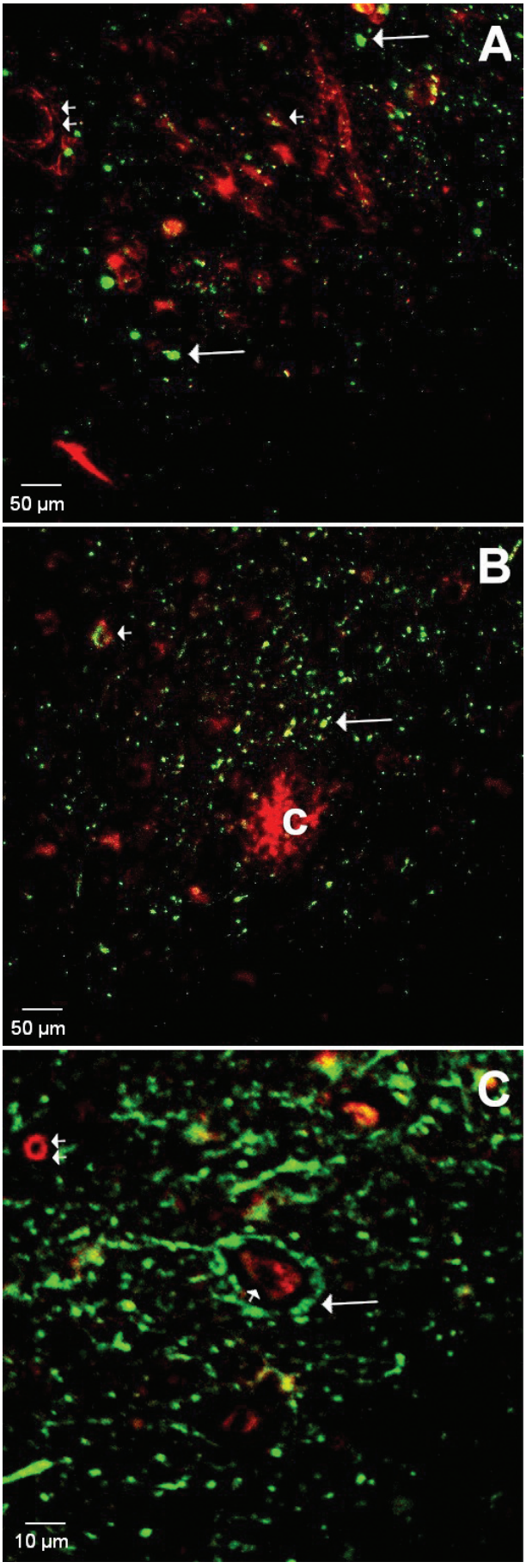

antibodies recognized several populations of dystrophic neurites and NFTs. However, AD2 antibody demonstrated to be highly specific for neuritic degeneration, including NFTs (not illustrated). TR also stained perfectly the neuritic degeneration and the NFTs observed with the antibody AD2. The neurofibrillary degeneration recognized by the mAb AD2 was seen in the green FITC channel and the amyloid-ß deposits in the red rhodamine channel. Figure 1 illustrates the four different lesions found in AD brains, which were characterized as: amyloid-ß deposits (diffuse senile plaques, AßDs) (Fig. 1C), classic neuritic plaques with an amyloid-ß core (NPs) (Fig. 1D), dystrophic neurites clusters lacking amyloid-ß (DNCs) (Fig. 1E), meganeuritic clusters immunoreactive to human synaptophysin (MCs) (Fig. 1F). These types of lesions were found in all the cortical regions analyzed in AD brains. The MCs were also recognized by the anti-tau antibodies (not illustrated) and were associated with amyloid-ß deposits (Fig. 1F).

Plaque density

Figure 3 shows the mean density (md) of lesions/mm^2 in the different cortical areas analyzed. AßDs, NPs, and DNCs were quantified in the same slides. AßDs were found in AD (md 2.29±0.7) and normal control brains (md 5.76±4.0). The AßDs were 2.5 times more abundant in normal control brains than in AD brains (Fig. 3A). Classic NPs were present in the AD brains (md 20.5±5.1) but less extended in the normal control brains (md 0.5±0.3) (Fig. 3A). The DNCs without amyloid-ß deposit were found in all regions of the cortex of AD brains (md 21.9±7.1) and, exceptionally, in one control brain (Fig. 3A). The mean density of MCs in the AD brains (md 2.2±0.3) was 10 times higher with respect to MCs in normal control brains (md 0.02±0.01) (Fig. 3A). Figure 2B illustrates the MCs immunoreactive to human synaptophysin. Among the 11 cortical regions analyzed, MCs were most abundant in regions 9 and 10.

Discussion

In this study, we demonstrated the co-existence of the main lesions characteristic of AD: AßDs, NPs, DNCs, MCs, and peri-neuronal sprouting. These lesions were observed at different density rates and distributions in the brains. AßDs were highly dense in normal control

Fig. 2. A. Meganeurites immunoreactive to GAP-43 (arrow). It is possible to observe incipient NFTs (arrowhead) and microvessels (double arrowhead). **B.** Meganeurites immunoreactive to GAP-43 (arrow). We found incipient NFTs (arrowhead). Note the GAP-43 in this neuron. amyloid-ß deposit (c). **C.** Double labeling for synaptophysin and TR in the hippocampus (region CA4). Meganeurites immunorective to synaptophysin (arrow) with and incipient NFT (arrowhead), and microvessels (double arrowhead) with amyloid-ß deposit.

brains and did not correlate with dementia. Our results suggest that, in normal elderly people, the extensive amyloid-ß deposits without cognitive impairment are related to the aging process per se (Davies et al., 1988; Delaère et al., 1990). NPs showed a good correlation with dementia in AD. These data are relevant since NPs are characterized by the addition of dystrophic neurites in amyloid-ß deposits. DNCs were found exclusively in AD brains, and yielded the best correlation with dementia. It has been suggested that DNCs might explain the cognitive deficits in AD patients (Barcikowska et al., 1989; Shin et al., 1989). DNCs observed in the extra-cellular space in AD brains could represent a massive aggregates of neurofibrillary degeneration process and dystrophic neurite fragmentation. Both dystrophic neurites and neurofibrillary tangles, might contribute to conform the NPs by they own aggregation around the insoluble amyloid-ß (Tabaton et al., 1989; Wisniewski et al., 1994). The aggregation process of insoluble proteins such as amyloid-ß and tau, among various proteins, is not well understood yet. Several post-translational modifications of these proteins might participate in this phenomenon (Grundke-Iqbal et al., 1986; Flament and Delacourte 1989; Ledesma et al., 1994; Yan et al., 1994; Smith et al., 1994, 1996; Wang et al., 1996; Guevara et al., 1998).

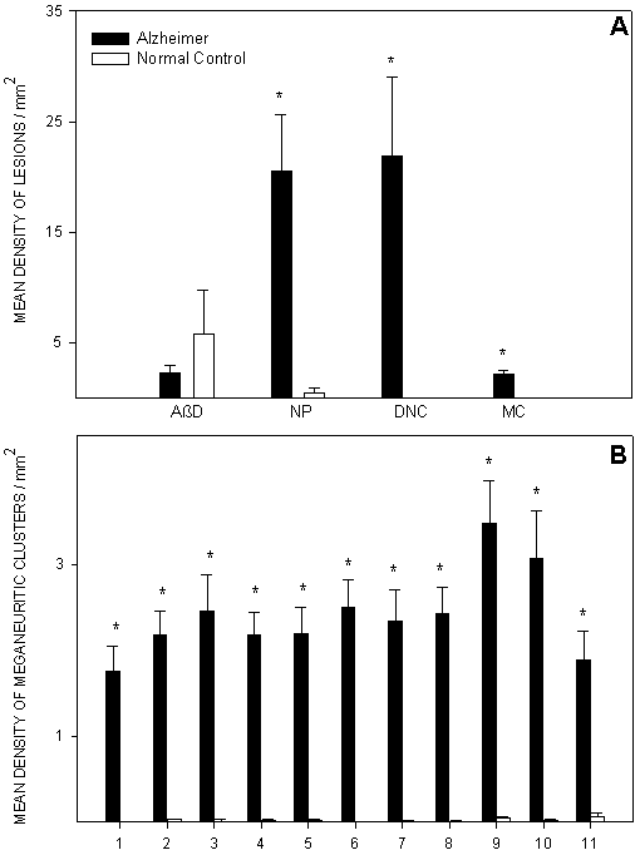

Fig. 3. **A**. Mean density of AßDs, NPs, DNCs, and MCs in AD and non-demented control brains evaluated by quantification of the different lesions in double labeled slides and through confocal microscopy. **B.** Mean density of meganeuritic clusters immunoreactive to human synaptophysin. Mean density of meganeuritic clusters was determined in 11 cortical brain regions (see methods). We found a significant difference for all the brain regions (*p>0.0001; two-tails Student's t-test).

We found reactive plasticity processes exclusively in AD brains, as demonstrated by the existence of two abnormally dilated synaptic terminals: peri-neuronal and MCs (meganeurites). The peri-neuronal synaptic terminals were observed specifically on CA4 neurons of the hippocampus from AD cases but not in all the controls. We suggest that synaptic plasticity might be linked to aging and a pathological perforant pathway deafferentation. The MCs were observed in all regions of the brain from AD cases but was not present in normal cases. Our results suggest that reactive plasticity, demonstrated by MCs and peri-neuronal synaptic terminals, as well as synaptogenesis, represent a regeneration process in AD brains. Coleman and Flood (1986) demonstrated that increased dendrite sprouting found in normal aging subjects, can also be observed in the remnant cortical neurons in AD. As indicated by immunostaining, GAP-43 was highly expressed in pathological regions but also in incipient NFTs, wherever they were found. In contrast, peri-neuronal sprouting immunoreactive to synaptophysin was observed mainly in incipient NFTs of CA4, and less frequently in brain cortex regions. We interpreted this result as an indicator of increased sprouting following lesions (Guevara et al., 1997). In fact, reactive plasticity was observed in all the regions analyzed from the brain-cortex. The markers tested in this work, i.e., synaptophysin and GAP-43, presented higher immunoreactivity in all brain areas of AD cases than in normal control brains. ApoE genotype has been associated to the synaptic pathology in Alzheimer disease. Since were not enough ApoE genotyped groups of patients for this study, we cannot reach any conclusion on this issue; however, considering that ApoE4 represented the highest population of the AD cases studied in this work, a direct link between synaptic sprouting and ApoE genotype might be suggested. More detailed studies must be performed to elucidate this issue.

The use of lectins specific for sialic acid and O-glycans permitted identifying a particular pattern of glycosylation in sprouting mechanisms, specifically of meganeurites in AD (Espinosa et al., 2001). The sprouting nature of the glycosylated meganeurites was confirmed by double-staining assays with lectins and TR, PHF-hyperphosphorylated tau (mAb AD2), and synaptophysin. The presence of O-glycosidically linked glycans (Galß1,3GalNAcα1-O-Ser/Thr and GalNAcα1-O-Ser/Thr), identified by ALL, has been considered an indicator of maturity or cell transformation in macrophages; moreover, O-glycosylated receptors seem

to participate in the organization of cytoskeleton (Maldonado et al., 1998). It could be proposed that O-glycosylated receptors in AD neurons might also participate in the organization of microtubules and microfilaments (Gorocica et al., 1998), as well as specific receptors for microglia. The interaction of O-glycosylated receptors from neurons might be considered a specific signal to microglia to release trophic factors involved in the formation of neurites (Griffin et al., 1998). Our results also showed changes on AD neurons in the sialylation pattern, which are not present in normal neurons, and which seems to contribute to the early neurofibrillary degeneration (Wang et al., 1996; Araujo et al., 1997; Guevara et al., 1998) possibly inducing modifications in cell-cell communication (Boland 1991; Araujo 1997; Tetaert et al., 2001). Sialylated gangliosides have been implicated in numerous cellular functions in developing nervous systems and it has been suggested that altered glycosylation correlates with periods of cell migration and axonal pathfinding, suggesting a modulator role of sialic acid derivatives on growth cone motility and adhesion (Araujo et al., 1997). Although, the lack of suitable markers for sprouting in the human brain, ALL and MRL lectins are useful and accurate tools for the study of changes in neurodegenerative diseases, or to pinpoint the biochemical events that underlie the reactive plasticity response in AD (Guevara et al., 1997, 1998; Espinosa et al., 2001).

Based on this study, we suggest that CDN structures represent the most important neuritic degenerative condition for the development of Alzheimer's disease dementia. However, a reactive plasticity process must be present in AD brains; neuronal plasticity and MCs might represent an important response to the massive neuronal death observed in AD.

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