Acta Neuropathol (2000) 99 : 352–357

REGULAR PAPER

Estrella Gómez-Tortosa · Kathy Newell
Michael C. Irizarry · Judith L. Sanders
Bradley T. Hyman

α-Synuclein immunoreactivity in dementia with Lewy bodies: morphological staging and comparison with ubiquitin immunostaining

Received: 18 May 1999 / Revised: 28 July 1999 / Accepted: 30 July 1999

Abstract α-Synuclein is a presynaptic protein recently identified as a specific component of Lewy bodies (LB) and Lewy neurites. The aim of this study was to assess the morphology and distribution of α-synuclein immunoreactivity in cases of dementia with LB (DLB), and to compare α-synuclein with ubiquitin immunostaining. We examined substantia nigra, paralimbic regions (entorhinal cortex, cingulate gyrus, insula and hippocampus), and neocortex (frontal and occipital association cortices) with double α-synuclein and ubiquitin immunostaining in 25 cases meeting neuropathological criteria for DLB. α-Synuclein immunostaining was more specific than ubiquitin immunostaining in that it differentiated LB from globose tangles. It was also slightly more sensitive, staining 4–5% more intracytoplasmic structures, especially diffuse α-synuclein deposits that were ubiquitin negative. In addition to LB, α-synuclein staining showed filiform and globose neurites in the substantia nigra, CA2–3 regions of the hippocampus, and entorhinal cortex. A spectrum of α-synuclein staining was seen in substantia nigra: from diffuse "cloud-like" inclusions to aggregated intracytoplasmic inclusions with variable ubiquitin staining to classic LB. We hypothesize that these represent different stages in LB formation.

Key words Lewy bodies · α-Synuclein · Ubiquitin · Dementia · Parkinson's disease

E. Gómez-Tortosa · K. Newell · M. C. Irizarry · J. L. Sanders
B. T. Hyman
Neurology Department, Massachusetts General Hospital, Charlestown, Massachusetts, USA

B. T. Hyman (✉)
Alzheimer's Research Unit,
Massachusetts General Hospital East 149 13th St., Room 6405, Charlestown, MA 02129, USA
e-mail: b_hyman@helix.mgh.harvard.edu,
Tel.: +1-617-7262299, Fax: +1-617-7265677

Introduction

Dementia with Lewy bodies (DLB) is a common cause of degenerative dementia. The neuropathological hallmark is the presence of Lewy bodies (LB) widely distributed throughout brain stem, paralimbic, and neocortical regions [7, 19, 21]. LB are known to contain more than 20 different proteins, including structural and cytosolic proteins, enzymes, and proteins elicited as a cellular response such as ubiquitin [2, 7, 22]. Until recently, immunostaining against ubiquitin has been the most commonly used and sensitive technique to recognize LB [18, 19]. However, ubiquitin lacks specificity because it is also present in other intracytoplasmic inclusions such as neurofibrillary tangles and Pick bodies. In the past year, a presynaptic protein called α-synuclein has been identified as a very specific component of LB and Lewy-related neurites [4, 15, 24–26]. Moreover, two mutations in the α-synuclein gene have been linked to autosomal dominant familial Parkinson's disease [17, 23]. These data suggest that abnormal aggregation of α-synuclein with other proteins may be at the core of LB formation.

In this study we have examined the morphology and have quantitated the regional distribution of α-synuclein immunoreactivity in several brain regions in patients with DLB, emphasizing a comparison of α-synuclein versus ubiquitin immunostaining. We found a consistent distribution of α-synuclein inclusions and different morphological and immunohistochemical patterns of aggregation which may represent progressive stages of LB formation.

Methods

We studied the brains of 25 DLB cases [19 male/6 female; age at death 78.6 ± 5.8 years (mean ± SD), range 63–87 years) and 6 age-matched controls, obtained from the Massachusetts Alzheimer's Disease Research Center Brain Bank, Boston. Average duration of the disease was 9.4 ± 4.5 years (range 3–17 years). All the brains had been fixed in 10% buffered formalin and routinely dissected according to a standardized protocol [28]. Tissue blocks were obtained from 18 areas, embedded in paraffin and sections cut at

8 μm. Sections were stained with hematoxylin and eosin, Congo red and the modified Bielschowsky method. A pathological diagnosis of diffuse LB disease or LB variant of Alzheimer's disease (AD) had been made in all the cases based on the presence of classic LB within the pigmented brain stem nuclei and similar eosinophilic inclusion bodies distributed throughout limbic and neocortical regions [19]. The degree of AD-related changes was assessed using CERAD neuritic plaque scores [20] and by assigning a Braak and Braak stage based on the distribution of neurofibrillary changes [3]. According to CERAD criteria, 2 cases had no AD changes, 6 were possible, 7 were probable and 10 were definite AD. Thirteen cases met criteria for a Braak and Braak stage I or II. Another 12 cases had paralimbic or neocortical tangles and met criteria for a Braak and Braak stages III–V. We assessed the following areas: substantia nigra at the level of the red nucleus, entorhinal cortex, cingulate gyrus, insula, hippocampus, medial frontal and associative occipital cortex. We did not examine the temporal and parietal cortical regions recommended by the Consortium on DLB [19] because these areas were not consistently available in these cases.

We performed double-label immunofluorescence staining with rabbit polyclonal ubiquitin antibody (1:200, Dako, Denmark), and the monoclonal antibodies against α-synuclein H3C (1:5000, courtesy of Dr. David Clayton, University of Illinois, Urbana, Ill.) or LB509 (1:10000, courtesy of Dr. Iwatsubo, University of Tokyo, Tokyo, Japan). Both are well-characterized antibodies against α-synuclein. H3C was raised in mice against the C terminus of canary synelfin [6] and LB509 was generated from purified LB [1]. Both antibodies were used to assess the morphology of the inclusions but the quantitative study was performed with H3C. The secondary antibodies were cy3 anti-rabbit (Jackson ImmunoResearch, West Grove, Pa.) and BODIPY anti-mouse (Molecular Probes, Eugene, Ore.). Negative control sections were incubated with no primary antibodies. The slides were mounted with 4′, 6-diamino-2-phenylindole (DAPI) aqueous medium (Vectashield, Vector, Calif.), which stains nuclei under UV illumination. In 7 cases the section of substantia nigra was also counterstained with hematoxylin and aqueous eosin (H&E).

α-Synuclein immunoreactivity was examined systematically throughout the whole selected area available on each slide under a 40x objective and compared with anti-ubiquitin staining. Quantitation of intracytoplasmic and neuritic LB was recorded by a Bioquant Image Analysis System with Stereology Package (Nashville, Tenn.). This program provides stereological overlays to record the different positions of each of the counted structures with x/y coordinates providing an accurate visual image of the distribution of LB throughout cortical layers. The total number of LB counted in each region was divided by the area assessed to obtain a LB density, expressed in LB/mm^2 of tissue. Extensive search for intranuclear inclusions was conducted under a 100× objective alternating α-synuclein and nuclear stains. Confocal microscope images were taken from the different structures using the MRC-1024 Bio-Rad laser confocal imaging system mounted on a Nikon TE400 microscope at an excitation wavelength of 568 nm and an emission wavelength of 605 nm for cy3, and a 488 nm excitation and a 522 nm emission wavelength for BODIPY.

Results

Substantia nigra

The substantia nigra showed the greatest morphological diversity of α-synuclein immunoreactive structures. These can broadly be classified as intracytoplasmic or neuritic inclusions. Intracytoplasmic inclusions reflected different patterns of α-synuclein aggregation (Fig. 1A–H). Numerous classic LB with the characteristic halo were observed in all cases and some nigral neurons showed multiple LB. All the classic LB demonstrated a consistent pattern of α-synuclein and ubiquitin double staining: α-synuclein antibodies (both H3C and LB509) showed a greater tendency to stain an outer rim, while ubiquitin was more likely to stain the core (Fig. 1H).

Pale bodies were also α-synuclein immunoreactive. Some neurons had intracytoplasmic synuclein inclusions, larger than classic LB, with defined borders but without halo, which could be identified as pale bodies when counterstained with H&E. These inclusions sometimes coexisted in the same neuron with classic LB. Their pattern of ubiquitin staining was quite variable. Sometimes ubiquitin immunoreactivity colocalized with synuclein staining. Rare neurons showed ubiquitin-positive punctate staining within or surrounding the α-synuclein deposit (Fig. 1E, F), and in others the pale bodies were ubiquitin negative (Fig. 1C).

Other neurons contained diffuse or "cloud-like" α-synuclein staining with ill-defined borders which were most frequently ubiquitin negative (Fig. 1A, B, G). When counterstained with H&E these neurons were quite normal or could only be recognized as having an inclusion because of some displacement of the pigment. Occasionally, this diffuse α-synuclein staining showed a more intense region of aggregation, suggestive of incipient LB formation (Fig. 1D). This variety of intracytoplasmic inclusions frequently coexisted within the substantia nigra of a single case, suggesting that they may represent different stages in the formation of LB.

In addition to these intracytoplasmic inclusions there were numerous filiform and globose neurites (Fig. 2A–C). Sometimes they had the appearance of rows of intraneuritic LB (without halo) or of the spheroid axons seen in neuroaxonal dystrophies (Fig. 2C). Neuritic inclusions had the same staining pattern as classic LB, double labeling for α-synuclein and ubiquitin, with the α-synuclein staining the outer rim and the ubiquitin staining the inner core (Fig. 2B).

The substantia nigra in control cases did not show any of these α-synuclein inclusions.

Limbic and paralimbic regions

Most of the α-synuclein immunoreactivity in hippocampus was in neurites (Lewy neurites) distributed consistently in the CA2 and CA3 regions (Fig. 2D). Adopting CERAD-style nomenclature, Lewy neurites were frequent in 16 cases, were few in 4 and absent in 5 cases. α-Synuclein and ubiquitin immunostaining usually colocalized in these neurites, but α-synuclein-positive neurites outnumbered those immunoreactive for ubiquitin. In contrast, the density of intracytoplasmic inclusions in the hippocampus was low. There were scattered LB in the CA4, CA3 and subicular cortex. In some cases a few pyramidal neurons in the CA2 and CA3 showed α-synuclein inclusions with ill-defined limits and irregular shapes (triangle or U shaped). Neurons in the dentate gyrus were usually spared. In the hippocampus, α-synuclein and ubiquitin

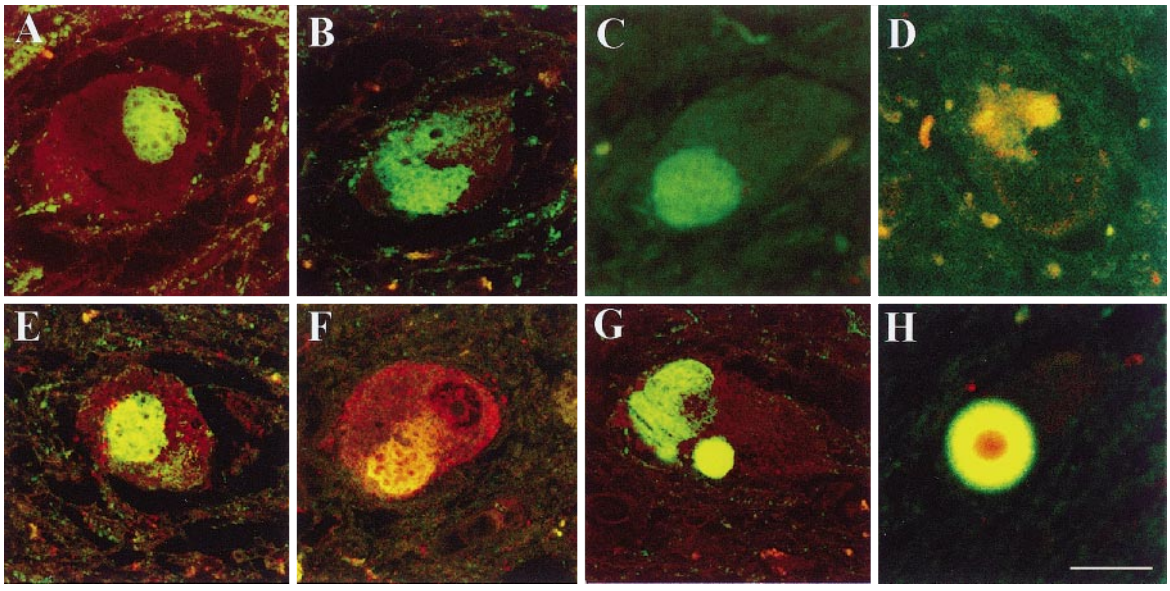

Fig. 1 A–H Confocal microscope images of substantia nigra of DLB cases with double immunostaining for α-synuclein (*green*) and ubiquitin (*red*); colocalization appears in *yellow*. There was a spectrum of α-synuclein intracytoplasmic inclusions with different morphology and variable ubiquitin immunostaining: from "cloud-like" (**A**,**B**,**D**,**G**), to well-defined inclusions resembling pale bodies (**C**), to classic Lewy bodies showing a peripheral rim immunoreactive for α-synuclein, a middle ring where α-synuclein and ubiquitin colocalized and a central core positive only for ubiquitin (**H**). Rare neurons showed α-synuclein deposits surrounded by a punctate ubiquitin-positive staining (**E**,**F**), or "cloud-like" inclusions which appeared to initiate a core of more compact aggregation (**D**). **A**, **B**, **D**–**G** H3C α-synuclein antibody, **C**, **H** LB509 α-synuclein antibody (*LB* Lewy bodies, *DLB* dementia with LB). *Bar* 10 μm

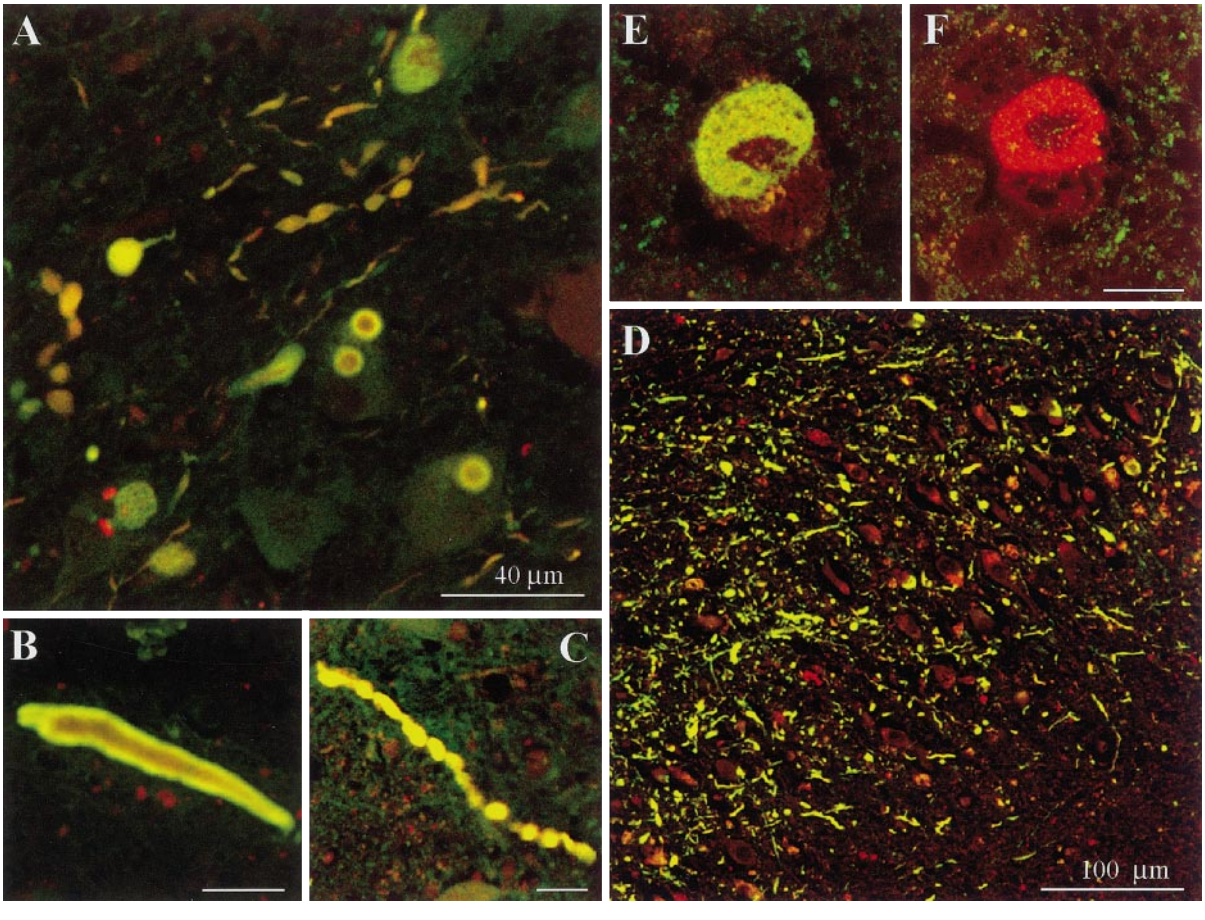

Table 1 Quantitative pathological data in the studied population. Data for LB represent mean ± SD (range) (*LB* Lewy bodies)

	n	LB/mm^2	Total counted (% ubiquitin negative)
Substantia nigra	25	3.15 ± 0.97 (1.43–5.02)	1720 (10.4)
Entorhinal cortex	21	1.70 ± 0.96 (0.42–4.73)	2054 (4.6)
Cingulate gyrus	24	1.37 ± 1.17 (0.08–5.55)	2614 (5.5)
Insula	18	1.26 ± 1.39 (0.03–5.51)	1055 (5.3)
Hippocampus	24	0.55 ± 0.54 (0.01–2.39)	664 (4.2)
Frontal cortex	24	0.62 ± 0.70 (0.04–2.93)	1316 (1.8)
Occipital cortex	24	0.15 ± 0.18 (0–0.77)	179 (1.6)

double immunostaining was extremely helpful in differentiating LB (positive for both immunostains, Fig. 2E) from globose tangles (only ubiquitin positive, Fig. 2F).

The entorhinal cortex had the highest density of intracytoplasmic inclusions of the cortical regions examined (Table 1). Cortical LB showed homogeneous round α-synuclein immunoreactivity, lacking the peripheral halo, and were clearly recognizable in deep layers (V and VI), with a well-defined laminar distribution. Half of the cases also showed intense α-synuclein immunoreactivity in superficial layers (II and III), within multiple filiform and globose neurites (with intraneuritic LB) and some cytoplasmic inclusions. Cingulate gyrus and insular cortex presented a similar pattern of α-synuclein immunoreactivity characterized by cortical LB (intracytoplasmic, round, quite well-defined inclusions without halo) with a laminar distribution within deep layers. LB showed a tendency to be clustered, and, especially in insular cortex, the distribution was irregular with some microarchitectural regions affected and others unaffected. Except for three cases with a very high density of LB pathology in paralimbic and cortical areas, neurites were rare.

In general, we observed two different patterns of LB distribution in paralimbic cortices. In one group of cases LB were clearly confined to deep layers, while in another they also spread to superficial layers. Each individual case usually displayed the same pattern in all paralimbic and neocortical areas. This pattern of "confined" versus "widespread" was not dependent on a higher LB density, a longer evolution of the disease or the presence of concomitant AD pathology. Many of the neurons with α-synuclein inclusions also contained autofluorescent lipofuscin. Limbic and paralimbic cortices of control cases did not show α-synuclein immunoreactivity.

◀**Fig. 2** Confocal microscope images of substantia nigra (**A**–**C**) and hippocampus (**D**–**F**) of DLB cases with double immunostaining for α-synuclein (*green*) and ubiquitin (*red*); colocalization appears in *yellow*. **A** The greatest diversity of α-synuclein inclusions was seen in substantia nigra. **B** Nigral neurites showed the same pattern of double staining as classic LB. **C** Some neurites resembled spheroid axons. **D** Lewy neurites were typical in the CA2–3 regions of the hippocampus. **E**,**F** A different pattern of immunostaining helped distinguish LB, both α-synuclein and ubiquitin positive (**E**) from globose tangles, only ubiquitin positive (**F**), in the hippocampus of the same case. **A**,**B** LB509 α-synuclein antibody, **C**–**F** H3C α-synuclein antibody. *Bars* **A** 40 μm; **B**, **C**, **E**, **F** 10 μm; **D** 100 μm

Neocortical regions

Medial frontal and occipital association areas had the lowest density of LB pathology (Table 1) with LB sparsely distributed within deep layers, demonstrating more than 98% ubiquitin colocalization. Most neocortical neurons containing LB also had autofluorescent lipofuscin. Three cases showed a very high density of α-synuclein inclusions in the neocortex, with LB that were much smaller than usual. Lewy neurites were rare. Diffuse accumulation of α-synuclein within the cytoplasm of cortical neurons was also uncommon; therefore, there were fewer ubiquitin-negative inclusions (less than 2%) than in substantia nigra or paralimbic areas. Neocortical regions of control cases did not show α-synuclein immunoreactivity.

Nuclear inclusions

We did not find nuclear inclusions with α-synuclein immunostaining in any of the regions.

Comparison with ubiquitin

α-Synuclein immunostaining qualitatively paralleled ubiquitin staining, but was more specific and slightly more sensitive, staining 4–5% more structures than ubiquitin. Around 10,000 intracytoplasmic inclusions were counted in the seven brain regions from the 25 cases. The percentage of structures that were α-synuclein positive but ubiquitin negative ranged from around 10% in substantia nigra, to 4–5% in paralimbic regions, to less than 2% in neocortex (Table 1). These ubiquitin negative inclusions corresponded to the less aggregated and less compact α-synuclein inclusions. Some were likely to be pale bodies and others did not even have defined borders and were just a "cloud-like" of α-synuclein immunoreactivity within the cytoplasm.

Discussion

The presence of aggregated α-synuclein immunoreactivity has recently been established as the basis for grouping several neurodegenerative disorders as "synucleinopathies" [8]. The group of LB disorders is characterized

by α-synuclein inclusions in neurons, while the multiple system atrophies are characterized for the presence of α-synuclein inclusions in glia [15, 24–27, 29]. In this study we examined α-synuclein immunoreactivity in multiple brain regions in cases with a neuropathological diagnosis of DLB, and compared this technique with ubiquitin immunostaining. These data validate α-synuclein immunocytochemistry as a sensitive and specific pathological technique for the study of LB disorders and further describe the morphological spectrum of inclusions in DLB.

α-Synuclein antibodies showed slightly higher sensitivity than ubiquitin antibodies for identifying abnormal inclusions, especially inclusions other than the classic LB, such as pale bodies or diffuse "cloud-like" inclusions. α-Synuclein immunostaining was also more specific than ubiquitin since it did not stain tangles. DLB presents frequently with concomitant AD changes [7, 21]. Although the distinct topographic and laminar distribution of LB and neurofibrillary tangles usually help distinguish these structures using ubiquitin immunostaining, it can be extremely difficult to distinguish LB from globose tangles in regions such as amygdala, entorhinal cortex, or hippocampus. In contrast, α-synuclein immunostaining was definitive for identifying LB. α-Synuclein antibodies also identified a wide array of neuritic inclusions, not always evident with ubiquitin, emphasizing that classic LB are just a part of Lewy pathology.

The morphological diversity of α-synuclein staining, best exemplified in the substantia nigra, may open new insights into the pathophysiology of LB. We hypothesize that the spectrum of α-synuclein inclusions with distinct morphological and ubiquitin staining characteristics represent different stages in the formation of LB and suggest a certain pattern of progression. First, there were some neurons with "cloud-like" α-synuclein staining, most frequently ubiquitin negative, that might be a very early stage in LB pathology. Such lesions would progress to more compact and better-defined α-synuclein inclusions that would begin attracting ubiquitin, thus showing variable ubiquitin immunolabeling. In fact, rare neurons showed a punctate ubiquitin immunostaining surrounding these α-synuclein conglomerates. Some of these inclusions could be recognized as pale bodies in H&E stain. Pale bodies are considered to be precursors of LB on the basis of their shared antigenicity, ultrastructure and co-occurrence in the substantia nigra in Parkinson's disease patients, and their ubiquitin immunolabeling is reported as variable [5, 10]. Eventually, the final product would be the classic LB with halo, showing a very compact, well defined and consistent pattern of double immunostaining, with α-synuclein staining an outer layer and ubiquitin the core.

This progression would be consistent with a role for α-synuclein as an early protein involved in LB pathology [9, 26], and the role of ubiquitin as part of the cellular response to target abnormal aggregation of proteins for subsequent proteolysis by the proteasome [12–14]. The incorporation of ubiquitin as a secondary event suggests that its differential detection within α-synuclein inclusions may be used as a clue for staging the inclusion bodies' biogenesis. The progression from a "cloud-like" α-synuclein deposit to the compact and laminar configuration of the double-labeled, typical haloed LB, known to be limited by a corona of radiating neurofilaments [11], may reflect the cell processing of abnormal proteins and, ultimately, inefficient proteolysis within the proteasome. In this scenario, LB may be comparable to aggresomes, a novel cytoplasmic inclusion which has recently been experimentally generated after proteasome inhibition [16]. Aggresomes are defined as cytoplasmic inclusions containing misfolded, ubiquitinated proteins ensheathed in a cage of intermediate neurofilaments which form specifically at the microtubule-organizing center.

In contrast to the diversity found in substantia nigra, neocortical and paralimbic α-synuclein immunoreactivity was more homogeneous in the morphology and ubiquitin staining of the inclusions within each individual region. The cortex contained mostly double-labeled LB without halo in deep layers, CA2–3 contained only Lewy neurites, and a subset of cases showed remarkable neuritic processes in superficial entorhinal layers. Furthermore, while the substantia nigra demonstrated α-synuclein pathology at all stages of aggregation, cortical inclusions appeared to be at the same stage of development. Whether cortical LB lack the peripheral halo typical of nigral LB because they represent a less mature stage of these inclusions remains unclear. This regional "pattern" specificity may be related to anatomic and/or biochemical neuronal vulnerability, including the specific connectivity patterns of the hippocampal CA2–3 region, or the susceptibility to oxidative stress of pigmented neurons or neurons containing lipofuscin.

In summary, our observations show that α-synuclein immunoreactivity has distinct regional patterns and that there are several different types of α-synuclein inclusions that can be distinguished by their morphology and their degree of ubiquitin immunoreactivity. Interpreted in the context of experimental data on ubiquitinization of abnormally folded proteins and subsequent proteasomal degradation [12, 13, 16], we suggest that these different types of inclusions represent different stages of LB formation.

Acknowledgements E. Gómez-Tortosa was supported by a grant from Fundación Ramón Areces, Spain. This work was supported by NIH grants P5O-AGO5134 (ADRC), AGO8487 and MGH-MIT Parkinson Center.

References

1. Baba M, Nakajo S, Tu P, Tomita T, Nakaya K, Lee VM-Y, Trojanowski JQ, Iwatsubo T (1998) Aggregation of α-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. Am J Pathol 152: 879–884
2. Bancher C, Lassmann H, Budka H, Jellinger K, Grundke-Iqbal I, Iqbal K, Wiche G, Seitelberger F, Wisniewski HM (1989) An antigenic profile of Lewy bodies: immunocytochemical indication for protein phosphorylation and ubiquitination. J Neuropathol Exp Neurol 48: 81–93
3. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82: 239–259

4. Clayton DF, George JM (1998) The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. Trends Neurosci 21: 249–254
5. Dale G, Probst A, Luthert P, Martin J, Anderton B, Leigh P (1992) Relationships between Lewy bodies and pale bodies in Parkinson's disease. Acta Neuropathol 83: 525–529
6. George J, Jin H, Woods W, Clayton D (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15: 361–372
7. Gómez-Tortosa E, Ingraham AO, Irizarry MC, Hyman BT (1998) Dementia with Lewy bodies. J Am Geriatr Soc 46: 1449–1458
8. Hardy J, Gwinn-Hardy K (1998) Genetic classification of primary neurodegenerative disease. Science 282: 1075–1079
9. Hashimoto M, Hsu LJ, Sisk A, Xia Y, Takeda A, Sundsmo M, Masliah E (1998) Human recombinant NACP/α-synuclein is aggregated and fibrillated in vitro: relevance for Lewy body disease. Brain Res 799: 301–306
10. Hayashida K, Oyanagi S, Mizutani Y, Yokochi M (1993) An early cytoplasmic change before Lewy body maturation: an ultrastructural study of the substantia nigra from an autopsied case of juvenile parkinsonism. Acta Neuropathol 85: 445–448
11. Hill WD, Lee VM-Y, Hurting HI, Murray JM, Trojanowski JQ (1991) Epitopes located in spatially separate domains of each neurofilament subunit are present in Parkinson's disease Lewy bodies. J Comp Neurol 309: 150–160
12. Hilt W, Wolf DH (1996) Proteasomes: destruction as a programme. Trends Biochem Sci 21: 96–102
13. Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Annu Rev Genet 30: 405–439
14. Ii K, Ito H, Tanaka K, Hirano A (1997) Immunocytochemical co-localization of the proteasome in ubiquitinated structures in neurodegenerative diseases and the elderly. J Neuropathol Exp Neurol 56: 125–131
15. Irizarry MC, Growdon W, Gómez-Isla T, Newell K, George JM, Clayton DF, Hyman BT (1998) Nigral and cortical Lewy bodies and dystrophic nigral neurites in Parkinson's disease and cortical Lewy body disease contain α-synuclein immunoreactivity. J Neuropathol Exp Neurol 57: 334–337
16. Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143: 1883–1898
17. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, Riess O (1998) Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. Nat Genet 18: 106–108
18. Kuzuhara S, Mori H, Izumiyama N, Yoshimura M, Ihara Y (1988) Lewy bodies are ubiquitinated. A light and electron microscope study. Acta Neuropathol (Berl) 75: 345–353
19. McKeith I, Galasko D, Kosaka K, et al (1996) Consensus guidelines for the clinical and pathological diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 47: 1113–1124
20. Mirra SS, Heyman A, McKeel D, et al (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
21. Perry R, McKeith I, Perry E (1996) Dementia with Lewy bodies. Clinical, pathological, and treatment issues. Cambridge University Press, Cambridge
22. Pollanen MS, Dickson DW, Bergeron C (1993) Pathology and biology of the Lewy body. J Neuropathol Exp Neurol 52: 183–191
23. Polymeropoulos MH, Lavedan C, Leroy E, et al (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 276: 2045–2047
24. Spillantini M, Schmidt M, Lee VM-Y, Trojanowski JQ, Jakes R, Goedert M (1997) α-Synuclein in Lewy bodies. Nature 388: 839–840
25. Takeda A, Mallory M, Sundsmo M, Honer W, Hansen L, Masliah E (1998) Abnormal accumulation of NACP/α-synuclein in neurodegenerative disorders. Am J Pathol 152: 367–372
26. Trojanowski JQ, Lee VM-Y (1998) Aggregation of neurofilament and α-synuclein proteins in Lewy bodies: implications for the pathogenesis of Parkinson disease and Lewy body dementia. Arch Neurol 55: 151–152
27. Tu P, Galvin JE, Baba M, Giasson B, Tomita T, Leight S, Nakajo S, Iwatsubo T, Trojanowski JQ, Lee VM-Y (1998) Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble α-synuclein. Ann Neurol 44: 415–422
28. Vonsattel JP, Aizawa H, Ge P, DiFiglia M, McKee AC, MacDonald M, Gusella JF, Landwehrmeyer BG, Bird ED, Richardson EP, Hedley-Whyte ET (1995) An improved approach to prepare human brains for research. J Neuropathol Exp Neurol 54: 42–56
29. Wakabayashi K, Hayashi S, Kakita A, Yamada M, Toyoshima Y, Yoshimoto M, Takahashi H (1998) Accumulation of α-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy. Acta Neuropathol 96: 445–452