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Fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis revealed by organelle-specific antibodies

(immunocytochemistry/morphometry/membrane polypeptide)

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ABSTRACT **Many studies have established the central involvement of the Golgi apparatus in the transport and processing of plasma membrane, lysosomal, and secreted proteins. The Golgi apparatus of neurons is also involved in the axoplasmic flow of fast-moving macromolecules and in the orthograde, retrograde, and transsynaptic transport of exogenous ligands. Markers of the Golgi apparatus, based on traditional methods of enzyme cytochemistry, are not applicable to human tissues obtained at autopsy. For that reason, the Golgi apparatus of brain cells has not been examined adequately in diseases of the human nervous system. Here we report that an antiserum raised against MG-160, a 160-kDa sialoglycoprotein of medial cisternae of the Golgi apparatus of several rat cells, is a specific and easily reproducible immunocytochemical marker of the Golgi apparatus of human neurons and other cells obtained at autopsy. Application of this probe in amyotrophic lateral sclerosis has shown a fragmentation of the Golgi apparatus in motor neurons similar to that induced by depolymerization of microtubules. We suggest that the fragmentation of the Golgi apparatus of motor neurons in amyotrophic lateral sclerosis has functional implications because significant reductions of secretion of insulin and immunoglobulins have been observed in islet cells and plasma cells, respectively, treated with microtubule-disrupting agents.**

Amyotrophic lateral sclerosis (ALS) is a devastating and incurable disease of progressive degeneration of motor neurons and atrophy of skeletal muscle (1). Sensory and high cortical functions are intact. The etiology of the disease is not known and treatment is not available. Among the proposed but not proven hypotheses concerning the etiology of the disease are the following: a lesion of skeletal muscle and/or end plate causing a retrograde atrophy of motor neurons, autoimmunity, viral infection, failure of a putative growth or maintenance factor, toxic agents, and aging (2–7).

The cellular and molecular aspects of the neuronal degeneration in ALS have not been examined adequately. Study of the neurons at risk in ALS with cellular and molecular probes may introduce insights into the pathogenesis of the disease.

Recently we isolated from rat brain an intrinsic membrane sialoglycoprotein of the Golgi apparatus of neurons and other cells of ≈160-kDa apparent molecular mass (MG-160) (8). A rabbit was immunized with MG-160, which was purified by immunoaffinity chromatography according to a previously published procedure (8). Immunoblots of a crude membrane fraction from human brain with the anti-MG-160 antiserum showed a prominent band of ≈140 kDa in apparent molecular mass (Fig. 1). By light microscopic immunocytochemistry performed in human tissues obtained at autopsy and embedded in paraffin, the antiserum reacted with linear, granular, or reticular structures consistent with the Golgi apparatus of human motor neurons, pyramidal neurons of hippocampus, Purkinje neurons of cerebellum, and several other nonneuronal cells (Fig. 2*A*). By ultrastructural immunocytochemistry performed in segments of temporal lobes resected from three patients with intractable epilepsy, the antiserum stained only the cisternae of the Golgi apparatus of neurons and glia (not illustrated) (9). In this report, the antiserum against the 140-kDa polypeptide of the Golgi apparatus was applied in a study of the organelle in motor neurons from the cervical segment of the spinal cord from three patients with ALS and three controls.

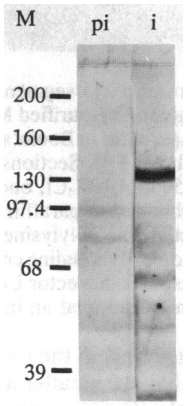

FIG. 1. Immunoblot of a crude membrane fraction from human brain, prepared as described (8, 9) and probed with an antiserum against MG-160. Seventy-five micrograms of the membrane fraction was electrophoresed according to Laemmli (10), and proteins were transferred to nitrocellulose according to Towbin *et al.* (11). Protein was measured according to Bradford (12). The antiserum was used in a 1:500 dilution in buffered physiologic saline. M, molecular mass markers (shown in kDa); pi, preimmune serum; i, immune serum.

A summary of the patients studied is presented in Table 1. The diagnosis of ALS was established during life and confirmed by neuropathologic examination. One patient with ALS had dementia (case 85-207); however, lesions of Alzheimer disease—i.e., senile plaques and neurofibrillary degeneration—or other histopathologic lesions were not found in this case. To investigate a possible effect of aging on the neuronal Golgi apparatus, two of the controls were 90 and 95 years old. In Fig. 2, motor neurons from an ALS patient and a control immunostained for the Golgi apparatus with the antiserum against the 140-kDa polypeptide are presented. In the control (Fig. 2*A*), the Golgi apparatus is in the form of round, oval, or irregularly shaped profiles that show a significant variation of surface areas; in contrast, the Golgi

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Abbreviation: ALS, amyotrophic lateral sclerosis.

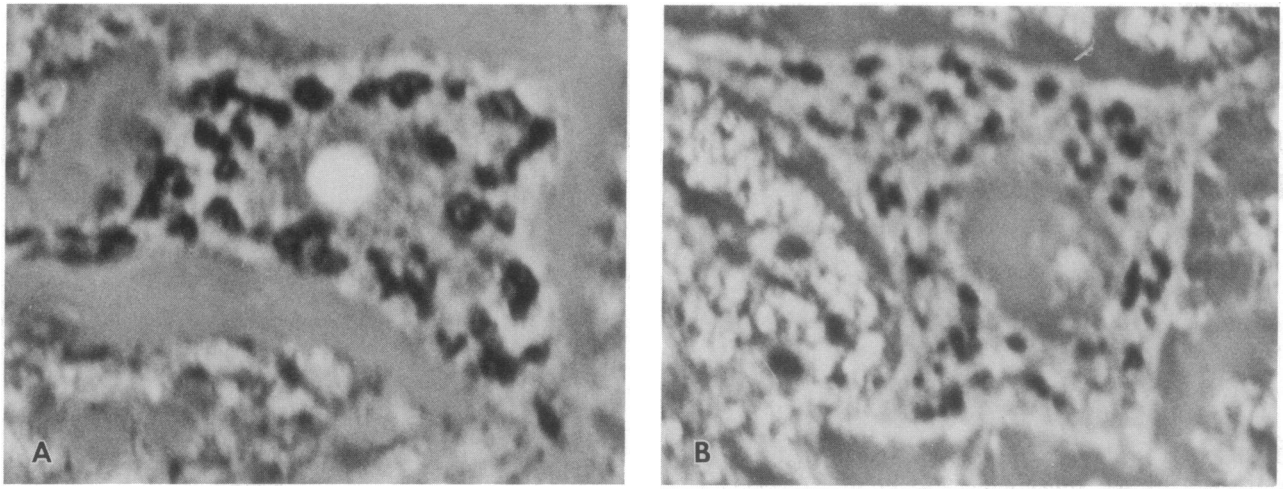

FIG. 2. Neurons from cervical segments 5–7 from a normal control (*A*, 89–140) and an ALS patient (*B*, 11/4/88) immunostained with antiserum against immunoaffinity-purified MG-160. Final magnification of *A* and *B* is ×3000. Transverse, 0.5-cm-thick slices of spinal cord were fixed for 6 hr at room temperature in Bouin's fixative [71% (vol/vol) saturated picric acid/24% (vol/vol) formaldehyde/5% (vol/vol) glacial acetic acid/0.7% (wt/vol) NaCl, pH 3.0]. Sections were then washed at room temperature thrice for 15 min each with phosphate-buffered physiologic saline (PBS) containing 50 mM NH_4Cl, once in PBS and overnight in PBS containing 50 mM NH_4Cl, and once in PBS and overnight in PBS at 4°C. Tissues were embedded in paraffin and 5-μm-thick serial sections were obtained. To avoid counting the same cell twice, every other fourth section was mounted on a polylysine-coated slide. Sections were immunostained as detailed (8). The primary antiserum against MG-160 was used in a 1:400 dilution of Tris/saline containing 10% normal goat serum. Goat anti-rabbit IgG and the ABC (avidin-biotinylated horseradish peroxidase) were purchased from Vector Laboratories and used according to the instructions of the vendor. Photomicrographs were taken with a Leitz phase-contrast microscope at an initial magnification of ×320.

apparatus in ALS (Fig. 2*B*) is in the form of numerous round or oval stained areas that are smaller and more uniform than the organelle in controls. The morphologic differences of the Golgi apparatus between ALS and controls were restricted to motor neurons. A survey of other neuronal groups in the spinal cord, such as the neurons of the nucleus of the column of Clarke, did not reveal any differences between ALS and controls. The fragmentation of the Golgi apparatus in ALS motor neurons was observed in cells retaining the usual polygonal shape and centrally placed nucleus (Fig. 2*B*); therefore, the changes of the Golgi apparatus cannot be attributed to chromatolysis that is associated with a round cytoplasmic profile and a peripherally displaced nucleus (13, 14).

In Table 2, the results of a morphometric study of the Golgi apparatus of motor neurons in ALS patients and controls are summarized. In ALS there is a significant reduction of the overall cell surface, an increase of the number of Golgi apparatus per cell, and a decrease of the total Golgi area per cell and the relative area occupied by the Golgi apparatus. Histograms of the number and surface areas occupied by apparently separate Golgi apparatus in ALS and controls showed significant differences. In ALS, there is a sharp peak of numbers of Golgi apparatus with a surface area of 0.5 μm^2; in controls, there is a broader distribution of surface areas of individual Golgi apparatus, whereas the largest number of Golgi apparatus has a surface area of 1–2 μm^2 (not illustrated here).

This study cannot answer the question whether the apparently discrete and separate Golgi apparatus in ALS and controls are indeed separate organelles or part of a continuous network interconnected with thin cisternae that are beyond the resolution power of this light microscopic immunocytochemical study. We have shown previously in thick sections of human fibroblasts examined in the electron microscope that the cisternae of the Golgi apparatus form a continuous network (15). A similar approach, if feasible in human tissues obtained at autopsy, may clarify the issue of whether the discrete elements of the Golgi apparatus of motor neurons in ALS are interconnected.

In a recent study, Turner and Tartakoff (16) have noted that microtubule depolymerization in bovine kidney cells is associated with a change of the Golgi apparatus from a reticular structure to numerous small granules. A similar fragmentation of the Golgi apparatus of ALS motor neurons was noted here (Fig. 2*B*). The reported fragmentation and reaggregation

Table 1. Summary of ALS patients and controls studied

	Age	Sex	Time between death and autopsy, hr	Cause of death	Significant information
Control					
88-187	90	♀	14	Aspiration-cardiopulmonary arrest	Food aspiration; pulmonary emboli; severe systemic atherosclerosis
89-58	54	♀	9	Aspiration pneumonia	Small cell carcinoma of lung
89-140	95	♂	11	Pulmonary embolization	Thromboembolus, right internal carotid and middle cerebral arteries
ALS					
85-207	56	♀	7.5	ALS	Duration, 12–18 months; dementia
88-108	87	♀	3	ALS	Duration, 2 years; rare neurofibrillary tangles in entorhinal cortex
11/4/88	86	♀	3	ALS	Duration, 6–8 months; senile changes in cerebral cortex

Table 2. Morphometric studies on the Golgi apparatus of motor neurons in ALS patients and controls

Parameter	Control	ALS
No. of cells studied	120	114
No. of nuclei studied	118	94
Areas of entire cell in μm^2*	144.97 ± 38.46	115.00 ± 16.00
Nuclear area per cell in μm^2*	16.07 ± 5.74	12.56 ± 5.75
Golgi area per cell in μm^2*	44.55 ± 13.24	18.26 ± 6.91
No. of Golgi apparatus per cell*	20.37 ± 6.64	39.96 ± 12.10
% of Golgi area per total cell area*	32.12 ± 4.49	16.48 ± 4.43

Photomicrographs were taken with a Leitz phase-contrast microscope at ×320 initial magnification and printed at a ×5000 final magnification. Panatomic X film was used. Morphometric analysis was done with a summagraphics morphometer connected to an IBM XT computer; the program was designed by Marc M. Friedman (Woods Hole Educational Associates).
*Data are expressed as mean ± SD.

of the Golgi apparatus (complex) following microtubule depolymerization–reformation is a multistep process dependent on energy and ionic composition and not determined by its binding to microtubules alone (16).

In ALS, and following axonotomy, dispersion of the Nissl substance (chromatolysis) has been described (13, 14). In a recent immunocytochemical study with an organelle-specific antibody of the Golgi apparatus in chromatolysis, we have confirmed the peripheral dispersion of the organelle (13). However, the marked fragmentation of the organelle observed in ALS was not seen in the Golgi apparatus of chromatolytic neurons (13). Therefore, it is unlikely that the fragmentation of the Golgi apparatus in ALS motor neurons is the result of chromatolysis.

Two of three controls were 90 and 95 years old. The Golgi apparatus of all neurons studied in these two controls did not show the fragmentation of the organelle found in the ALS neurons. Therefore, aging is probably not the cause of the fragmentation of Golgi apparatus.

Denervation affects the distribution of the Golgi apparatus in skeletal muscle, and it is possible that the observed "lesion" of the Golgi apparatus of motor neurons in ALS is secondary to deafferentation and atrophy of these cells (17).

The data suggest that the decrease in size and fragmentation of the Golgi apparatus in ALS is due to a lesion affecting the linkage of the organelle with microtubules. The similarities between the observed fragmentation of the Golgi apparatus in ALS and in experiments with microtubule-disrupting agents form the basis of the hypothesis (16, 18, 19). The Golgi apparatus plays a key role in the processing and transport of plasma membrane, secreted and lysosomal proteins (20). In neurons, the Golgi apparatus is involved in axoplasmic flow including the orthograde, retrograde, and transsynaptic transport of exogenous macromolecules (21–23). Furthermore, microtubule disruption affects secretion (24, 25). For these reasons, we believe that the observed fragmentation of the neuronal Golgi apparatus in ALS has significant functional implications. The observed lesion of the Golgi apparatus in motor neurons in ALS is, so far, unique in human disease. Probing of the neuronal Golgi apparatus and other cytoplasmic constituents in ALS, such as microtubules, as well as experimental studies on the effect of microtubule-depolymerizing agents or deafferentation on the neuronal Golgi apparatus may validate this hypothesis.

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