NOTES

Accumulation of Acid-Fast Lipochrome Bodies in Glial Cells of the Midbrain Nigral Lesion in Parkinson’s Disease

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The cause of Parkinson’s disease (PD) remains unknown. PD is probably caused by an environmental agent rather than a hereditary factor, but heredity may play a role in the vulnerability of certain individuals to the environmental agent (3, 20). Nocardia asteroides, a soil-borne acid-fast bacterium, causes movement disorder in laboratory animals. This movement disorder, which is clinically and pathologically similar to PD, emerges late after the elimination of filamentous nocardiae from the brain (19). Besides filamentous forms, N. asteroides spontaneously produces filterable, cell wall-defective forms. A possible role for filterable nocardiae is suspected in the progression of the PD-like movement disorder (15). Soil-borne nocardiae have been a suspected cause of PD. However, the results of serological testing do not support the hypothesis that nocardiae cause PD (13, 18). The proof of this hypothesis may require a reliable means of detecting nocardiae in postmortem brain tissues. PD is pathologically characterized by neuronal loss, reactive gliosis, and Lewy bodies in remaining neurons at the pars compacta of the midbrain substantia nigra (4, 11, 21). Filterable nocardiae are gram negative and acid fast and have a granular to spherical shape by nomarski optics (15). We investigated the presence of acid-fast spherical structures similar to filterable nocardiae at the midbrain nigral lesions of three patients with PD. Many clusters of acid-fast lipochrome bodies were dense around blood vessels in the two patients with Hoehn and Yahr stage II and III PD. These clusters were present in the vicinity of melanin-pigmented neurons in the three PD patients studied. Examination of adjacent hematoxylin-and-eosin-stained sections indicated that they consisted of yellow-green granules, bodies, and aggregates in ballooned glial cells. On the other hand, no clusters of acid-fast lipochrome bodies were observed at the compacta region of three control patients. Our results suggest that the immunological and genetic relationship between the acid-fast lipochrome bodies and filterable nocardiae should be investigated.

The cause of Parkinson’s disease (PD) remains unknown. PD is probably caused by an environmental agent rather than a hereditary factor, but heredity may play a role in the vulnerability of certain individuals to the environmental agent (3, 20). N. asteroides, a soil-borne acid-fast bacterium, causes movement disorder in laboratory animals. This movement disorder, which is clinically and pathologically similar to PD, emerges late after the elimination of filamentous nocardiae from the brain (19). Besides filamentous forms, N. asteroides spontaneously produces filterable, cell wall-defective forms. A possible role for filterable nocardiae is suspected in the progression of the PD-like movement disorder (15). Soil-borne nocardiae have been a suspected cause of PD. However, the results of serological testing do not support the hypothesis that nocardiae cause PD (13, 18). The proof of this hypothesis may require a reliable means of detecting nocardiae in postmortem brain tissues. PD is pathologically characterized by neuronal loss, reactive gliosis, and Lewy bodies in remaining neurons at the pars compacta of the midbrain substantia nigra (4, 11, 21). Filterable nocardiae are gram negative and acid fast and have a granular to spherical shape by nomarski optics (15). We investigated the presence of acid-fast spherical structures similar to filterable nocardiae in the midbrain nigral lesions that occur with PD.

Patients and methods. Three patients with PD (aged 65 to 68 years; median age, 64 years) and three patients without neurologic disorder (aged 60 to 70 years; median age, 64 years), serving as age-matched controls, were selected from the archives of the Department of Pathology, Chubu National Hospital (Aichi, Japan), Nagano Red-Cross Hospital (Nagano, Japan), and Fuji National Hospital (Shizuoka, Japan). Informed consent was obtained from the patients and their close family members. The progression of PD (12) in the three patients, ranging from Hoehn and Yahr stages II to V, was assessed as follows. Patient 1 (male; 65 years old; symptom duration, 2 years) had stage II PD, patient 2 (male; 68 years old; symptom duration, 9 years) had stage III PD, and patient 3 (female; 68 years old; symptom duration, 19 years) had stage V PD. The diagnosis of PD was made by neurologists on the basis of the following results of neurological examinations. Patients have resting tremors and at least two of the following symptoms: (i) akinesia or bradykinesia, (ii) rigidity, or (iii) postural abnormalities. The disease shows unilateral onset and development, has a good to excellent response to L-dopa, and lacks definite encephalitis lethargica history, Alzheimer-type dementia, prominent autonomic symptoms, oculogyric crisis, cerebellar signs, or pyramidal signs (9). At autopsy, the brains obtained were fixed in 10% buffered formalin, after which blocks of tissue were excited and embedded in paraffin. Lewy bodies were always present in the substantia nigra. The midbrain block was serially sectioned at 5 μm. After dewaxing and rehydration, carbol-fuchsin was applied to the section slides for 5 min at room temperature. Destaining was done with 1% concentrated hydrochloric acid in 70% ethanol (vol/vol) for 5 min at room temperature (15, 17). No counterstain was used. The glycerol-mounted sections were observed with a Nikon Optiphot microscope with differential interference-contrast (nomarski) optics before and after acid-fast staining (15). Both tissue sections adjacent to the acid-fast stained sections were stained with hematoxylin and eosin (H&E). The carbol-fuchsin cold stain method was applied to some tissue sections adjacent to H&E-stained sections. Each slide was examined under a light microscope.

Results. A comparison with the substantia nigra of age-matched controls showed that numerous melanin-pigmented neurons were absent from the patients with PD (Fig. 1a and b).
FIG. 1. Light micrographs of H&E-stained sections at the pars compacta of the substantia nigra. (a) Many melanin-pigmented neurons were seen at the central area of the pars compacta in the control patient. N, nucleus of melanin-pigmented neuron; D, dorsal; V, ventral. Bar in panels a through c = 100 μm. Bar in panels d through f = 10 μm.

(b) In patient 1 (stage II), a few melanin-pigmented neurons are evident (similar to panel a), and many glial cells (arrowhead) can be seen. (c) Eosinophilic intracytoplasmic inclusion body in melanin-pigmented neurons. (d) In patient 2 (stage III), eosinophilic laminae of alternating densities surround a central core in probable melanin-pigmented neurons. (e) In patient 3 (stage IV), many eosinophilic intracytoplasmic inclusion bodies can be seen. (f) In patient 4 (stage V), no melanin-pigmented neurons are evident (similar to panel d). N, nucleus of melanin-pigmented neuron; D, dorsal; V, ventral. Bars in panels a through f = 10 μm.
FIG. 2. Light micrographs, by Nomarski optics, of acid-fast-stained sections of the pars compacta of the substantia nigra. (a) In patient 1 (stage II), many clusters of red-stained lipochrome bodies (large arrowhead) of various sizes are evident. (b) In patient 2 (stage III), clusters of red-stained lipochrome bodies (large arrowhead) are visible in some melanin-pigmented neurons. (c) Inclusion body (small arrowhead) in probable melanin-pigmented neuron. V, blood vessel. Bar = 5 μm. Magnification is the same for all panels.
FIG. 3. Light micrographs of H&E-stained sections at the pars compacta of the substantia nigra. (a) In patient 1 (stage II), many yellow-green aggregates and bodies in a ballooned glial cell (large arrowhead) can be seen. A small arrow indicates the nucleus of the glial cell. (b) Many yellow-green granules (large arrowhead) are evident in a ballooned glial cell. (c) Yellow-green aggregates and bodies (small arrows) in glial cells. (d) In patient 2 (stage III), a yellow-green aggregate (large arrowhead) is seen in a ballooned glial cell. (e) Many yellow-green granules (large arrowhead) were observed in a ballooned glial cell. (f) Yellow-green aggregates and bodies (small arrow) in a probable glial cell. (g) In patient 3 (stage V), many yellow-green granules (large arrowhead) are seen in a glial cell. (h and i) Many yellow-green granules and bodies (small arrows) in ballooned glial cells. C, blood capillary. Bar 5 μm. Magnification is the same for all panels.
Severe neuronal loss at the pars compacta had occurred in all three PD patients. In patient I (stage II), many glial cells were seen near blood vessels (Fig. 1c). Eosinophilic, intracytoplasmic inclusion bodies were seen in melanin-pigmented neurons (Fig. 1d and f) in the three PD patients. As shown in Fig. 1e, eosinophilic laminae of various densities, surrounding a central core, were observed within a probable melanin-pigmented neuron in patient 2. Many clusters of red-stained lipochrome bodies were seen by nomarski optics when acid-fast stain was applied. As shown in Fig. 2a and d, these clusters were dense in the perivascular regions of patients with stages II and III PD but not in that of the patient with stage V PD. Clusters of red-stained lipochrome bodies were commonly seen in the vicinity of the melanin-pigmented neurons in all three patients with PD. The size of the clusters appeared to be similar to that of the melanin granules, many of which were brown (Fig. 2b, e, and g). Examination of adjacent acid-fast-stained sections in the areas corresponding to the inclusion bodies, shown in Fig. 1d through f, indicated that the inclusion bodies were not stained (as indicated by small arrowheads in Fig. 2c, f, and h). No clusters of red-stained lipochrome bodies were observed at the compacta regions in the control patients (Fig. 2i). There were no red-stained granules in nonpigmented neurons (Fig. 2e and i). Examination of adjacent H&E-stained sections in the corresponding areas (large arrowheads) indicated that (i) clusters of acid-fast lipochrome bodies consisted of many yellow-green aggregates and bodies in ballooned glial cells (Fig. 2 and 3a, and Fig. 2d to i and Fig. 3d) and that (ii) acid-fast, granular lipochrome bodies consisted of yellow-green granules in ballooned glial cells (Fig. 2b to i and Fig. 3a and b, Fig. 2e to i and Fig. 3a to e, and Fig. 2g to i and Fig. 3a to g). When acid-fast stain was applied to the adjacent sections, the areas corresponding to Fig. 3c, f, h, and i showed acid-fast lipochrome bodies. Some of yellow-green bodies were partially eosinophilic (small arrows, Fig. 3c and i). Their color differed from that of melanin granules, which were brown (Fig. 3b and e). As shown in Fig. 3a, the nuclei of glial cells were often displaced to the periphery.

Discussion. Carbol-fuchsin is the principal dye used for the Zielh-Neelsen method and its modifications. Acid fastness depends on the ability of microorganisms to retain dye, even when they are treated with acid-alcohol solution. Fite et al. recommend the cold stain method for demonstrating acid-fast bacilli on paraffin sections (8). The cold stain method (with a minor modification) indicates that nocardiae are acid fast (15, 17). Based on studies by Duffy and Tennyson (6) and Gibb and Lees (10), eosinophilic, intracytoplasmic inclusion bodies, shown in Fig. 1d and f, were identical to the Lewy body. Laminae of various densities surrounding a central core, shown in Fig. 1e and 2f, were identical to the classical form of the Lewy body. The classical Zielh-Neelsen hot stain method sometimes shows the central core to be acid fast (4). The cold stain method showed that Lewy bodies and the central core were not acid fast. The specificity might be reduced by treatment with heat. As shown in Fig. 3, the acid-fast lipochrome bodies consisted of yellow-green granules, aggregates, and bodies in glial cells, ranging from granular types, similar in size to melanin granules, to nearly 5 μm in diameter. The yellow-green body, partially eosinophilic, was evident in many glial cells (Fig. 3c and i). The nuclei of glial cells were often displaced at the periphery (Fig. 3a), perhaps due to the yellow-green granules and bodies which appear to accumulate in glial cells. Scarpie-associated prion protein also accumulates in astrocytes during scrapie infection (5). The yellow-green bodies might be the infectious agent itself or pathological byproducts of endogenous origin. They differ from many melanin granules. Lipofuscin granules are not acid fast in brains of patients without neurologic disease (1). Red-stained granules were not observed in nonpigmented neurons of control patients (Fig. 2i). Duffy and Tennyson reported that lipofuscin in PD patients is red when stained by the classical Zielh-Neelsen method (6). As shown in Fig. 2e, red-stained granules were not evident in nonpigmented neurons at the dorsolateral area, where many melanin-pigmented neurons were observed. On the other hand, pink-stained spherical bodies were seen in a few nonpigmented neurons at the ventral area, where melanin-pigmented neurons were sparse. Lipofuscin granules seem to differ in size from the pink-stained spherical bodies, which might correspond to the lipofuscin described by Duffy and Tennyson (6). Experimental infection with filterable nocardiae yielded the following results. (i) Laboratory animals appeared to be healthy and to move as quickly as control animals after 4 months. (ii) Early in the fifth month after infection, laboratory animals appeared to move slowly. When their tails were picked up, the animals showed a peculiar style, similar to that of sleeping bats, and no hemiparesis. (iii) Neuronal loss at the pars compacta became evident late in the third month postinoculation. (iv) Inoculated organisms were present as acid-fast spherical bodies in glial cells around midbrain blood vessels (14, 16). Nocardiae enter the midbrain glial cells and neurons (2). These results suggest that filterable nocardiae may be potentially neuroinvasive. The acid-fast lipochrome bodies in the nigral lesions might be filterable nocardiae. Immunological and genetic identification of them is under way. There were many neurons but no acid-fast lipochrome bodies at the pars compacta of control patients. Neuronal losses were evident in the three PD patients studied. The acid-fast lipochrome bodies were dense in the early stages of PD but not at the end. Loss of nigral neurons is greatest at the beginning of PD (7). These findings suggest that the acid-fast lipochrome bodies might have been involved in the loss of nigral neurons in the three PD patients. The investigation of this possibility requires further study of patients in early stages of PD.

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