with MG132 (Fig. 3G: ~8% of inclusion-positive cells and H: ~12% of inclusion-positive cells). The inclusions were ~10 μm in diameter, being very similar in size to the neuronal cytoplasmic inclusions found in patients with FTLD-U [2].

3.3. Immunoblot analysis of intracellular inclusions in the cultured cell models

Cells expressing wild-type or mutant TDP-43 were sequentially extracted, and the supernatants and pellets were analyzed by immunoblotting. On analysis of cell lysates using anti-TDP-43 antibody, endogenous TDP-43 at 43 kDa was detected in all fractions. Immunoreactivity of it was the strongest in TX-soluble fraction, and was the weakest in Sar-insoluble fraction (black-lined arrowheads in Fig. 4A and C). A similar band pattern but with stronger immunoreactivities was detected in cells transfected with wild-type TDP-43 (WT).

The ΔNLS mutant TDP-43, which was detected as bands with slightly lower molecular weight than that of the wild-type, was also recovered mostly in the TS- and TX-soluble fractions (Fig. 4A). The intensities of bands in the Sar-soluble and -insoluble fractions were slightly increased after MG132 treatment (Fig. 4C). Interestingly, these were positive for anti-pS409/410 (a black arrowhead in Fig. 4D), suggesting that inhibition of proteasome activity induces the aggregation of phosphorylated ΔNLS.

In contrast, expression of Δ187–192 mutant TDP-43, which was detected as a band with almost the same molecular weight as that of endogenous TDP-43, resulted in significant increases in the Sar-soluble and -insoluble (ppt) fractions as compared with those in cells transfected with wild-type TDP-43 (Fig. 4A). Smeared and higher-molecular-weight bands were also detected in the Sar-soluble and -insoluble fractions (Fig. 4A). We also observed pS409/410-positive bands in the Sar-soluble and -insoluble fractions in the absence or presence of MG132 (white arrowheads in Fig. 4B and D).

Finally, immunoblots of lysates from cells expressing ΔNLS&187–192 mutant TDP-43 (~41 kDa bands marked with grey arrowheads in Fig. 4A and C) showed high-molecular-weight bands of ~45 kDa (black arrowheads in Fig. 4A and C) and smears in the Sar-soluble and -insoluble fractions. The bands of ~45 kDa, smears, and C-terminal fragments at 25–37 kDa were highly immunoreactive with anti-pS409/410 antibody independently of MG132 treatment (black-lined arrowheads in Fig. 4B and D). These were similar characteristic band patterns to those found in immunoblot analyses of brain lysates of FTLD-U and ALS as previously reported [6].

3.4. Deletion mutants of TDP-43 lost the exon skipping activity

To evaluate the functional significance of the deletion mutants of TDP-43 used in this study, we performed CFTR exon 9 skipping assay. As shown in Fig. 5B, mRNA from cells transfected with empty vector pcDNA3 gave only one RT-PCR band of 360 bp, while that from cells transfected with pcDNA3-TDP-43 wild-type gave two RT-PCR bands, 360 and 177 bp, showing that skipping of CFTR exon 9 was increased by expression of wild-type TDP-43. In contrast, only one RT-PCR band of 360 bp was observed from cells co-transfected with ΔNLS, Δ187–192, or ΔNLS&187–192, indicating that these mutants do not have skipping activity of CFTR exon 9, which is one of known physiological functions of TDP-43.

4. Discussion

In this study, using SH-SY5Y cells and a phosphorylated TDP-43 specific antibody established by ourselves, we examined the effect of deletion of two candidate sequences for NLS, residues 78–84 and 187–192 of TDP-43, and proteasomal inhibition on inclusion formation. Mislocalization of TDP-43 into cytoplasm caused by deletion of residues 78–84 proves that this sequence indeed functions as NLS. This result is largely consistent with the previous report by Winton et al., which showed that residues 82–98 were

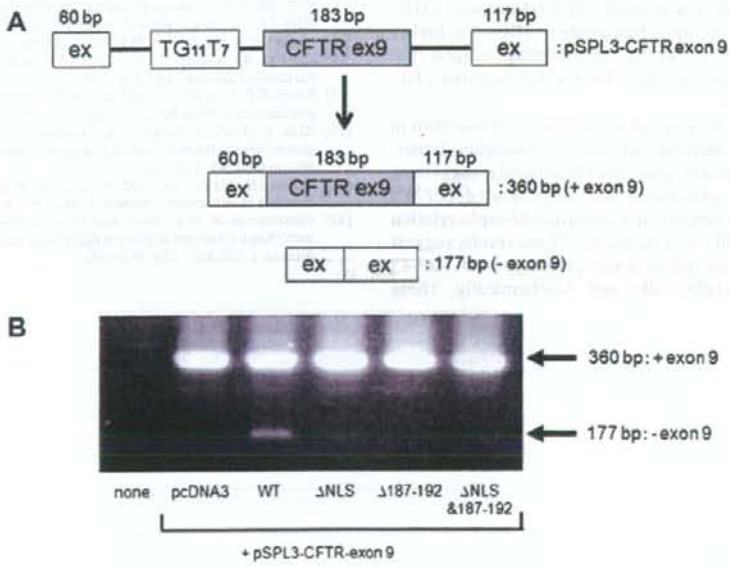


Fig. 5. CFTR exon 9 skipping assay of the deletion mutants of TDP-43. (A) Schematic diagram of the reporter plasmid pSPL3-CFTR exon 9. This plasmid contains the repeat sequence of TG11T7 in which the TG11 repeat is recognized by TDP-43, causing the CFTR exon 9 to be spliced out. The insert of this plasmid contains two exons of HIV-1 tat gene (60 and 117 bp, respectively: light grey boxes) flanking CFTR exon 9 (183 bp: a dark grey box). RT-PCR is expected to generate two products with (360 bp) and without CFTR exon 9 (177 bp). (B) Gel electrophoresis of RT-PCR products of RNA from transfected cos-7 cells. The RNAs from cos-7 cells, co-transfected with the reporter plasmid pSPL3-CFTR exon 9 plus pcDNA3 expression vectors were used as templates for RT-PCR analysis. The products were analyzed by electrophoresis in 1.5% agarose gel.

required for TDP-43 entry into the nucleus [7]. Formation of intranuclear TDP-43 positive dot-like structures caused by deletion of residues 187–192 suggests that this sequence does not function as a NLS but is nonetheless important to maintain a physiological state of TDP-43 in the nucleus. Loss of the exon skipping activity of CFTR exon 9 observed in cells transfected with this mutant may also support such a notion.

The results of the present study suggest that mislocalization of TDP-43 in cytoplasm is not a sufficient condition for aggregation of TDP-43, since the treatment of MG132, a proteasomal inhibitor, is needed to cause the formation of inclusion in cells transfected with a mutant TDP-43 lacking residues 78–84. Proteasome inhibition also induced formation of intranuclear inclusions in cells transfected with a mutant TDP-43 lacking residues 187–192. These results also suggest that a proteasome activity plays an important role for degradation of TDP-43. Impairment of the ubiquitin-proteasome system has recently been suggested to be related to the onset of neurodegenerative diseases. For instance, Bence et al reported that intracellular aggregates of a huntingtin fragment containing a pathogenic polyglutamine repeat directly impaired the function of the ubiquitin-proteasome system [9]. Keck et al., showed that proteasome was inhibited by paired helical filament-tau in brains of patients with Alzheimer's disease [10]. It should be further investigated whether the proteasome activity is actually decreased in brains of patients with TDP-43 proteinopathies, as Keller et al., reported that a significant decrease in proteasome activity was observed in AD brains [11]. Function of autophagy-lysosome degradation system may be an issue to be investigated as well, since inhibition of autophagic degradation by depletion of the endosomal sorting complexes required for transport (ESCRT) subunits causes accumulation of TDP-43 in ubiquitinated inclusions in cultured cells [12].

In contrast to a mutant lacking residues 78–84 or 187–192, double-deletion mutant of these sequences caused inclusion formation without proteasomal inhibition in this study. These results suggest the possibility that the double mutant protein has a higher propensity to aggregate than each single mutant protein. In this context, it should be noted that in insoluble fraction from FTLD-U brains, the amount of C-terminal fragments of TDP-43 is higher than that of the full-length TDP-43 [6]. These findings suggest that conformation or modifications of TDP-43 is another important factor for inclusion formation.

Importantly, intranuclear or cytoplasmic inclusions observed in this study were immunopositive for both a phosphorylation-dependent anti-TDP-43 antibody and anti-Ub antibody, suggesting that those consist of phosphorylated and ubiquitinated TDP-43. Biochemical analyses also support that abnormal phosphorylation of TDP-43 takes place in cells with inclusions. These results suggest that our cellular models recapitulate the phenotypes of TDP-43 proteinopathies both pathologically and biochemically. These models are expected to be valuable tools for understanding the pathological process underlying TDP-43 proteinopathies and for identifying candidate drugs to prevent intracellular aggregation of TDP-43.

Acknowledgements

We thank Dr. M. Arai (Tokyo Institute of Psychiatry) for providing human genomic DNA, and Drs. H. Mimuro (University of Tokyo) and F. Kametani (Tokyo Institute of Psychiatry) for helpful advice and discussions. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas-Research on Pathomechanisms of Brain Disorders (to M.H., 20023038) and Grants-in-Aid for Scientific Research (B) (to M.H., 18300117) and (C) (to T.N., 19590297 and T.A., 19591024) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2008.12.031.

References

[1] Snowden, J.S., Neary, D. and Mann, D.M. (2002) Frontotemporal dementia. Brit. J. Psychiatr. 180, 140–143.
[2] Arai, T. et al. (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem. Biophys. Res. Commun. 351, 602–611.
[3] Neumann, M. et al. (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.
[4] Ou, S.H., Wu, F., Harrich, D., Garcia-Martinez, L.F. and Gaynor, R.B. (1995) Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. J. Virol. 69, 3584–3596.
[5] Buratti, E., Dork, T., Zuccato, E., Pagani, F., Romano, M. and Baralle, F.E. (2001) Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J. 20, 1774–1784.
[6] Hasegawa, M. et al. (2008) Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann. Neurol. 64, 60–70.
[7] Winton, M.J., Igaz, L.M., Wong, M.M., Kwong, L.K., Trojanowski, J.Q. and Lee, V.M. (2008) Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. J. Biol. Chem. 283, 13302–13309.
[8] Glasson, B.I. and Lee, V.M. (2003) Are ubiquitination pathways central to Parkinson's disease? Cell 114, 1–8.
[9] Bence, N.F., Sampat, R.M. and Kopito, R.R. (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292, 1552–1555.
[10] Keck, S., Nitsch, R., Grune, T. and Ullrich, O. (2003) Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. J. Neurochem. 85, 115–122.
[11] Keller, J.N., Hanni, K.B. and Markesbery, W.R. (2000) Impaired proteasome function in Alzheimer's disease. J. Neurochem. 75, 436–439.
[12] Filimonenko, M. et al. (2007) Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J. Cell Biol. 179, 485–500.

Acta Neuropathol
DOI 10.1007/s00401-008-0460-5

EDITORIAL

Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations

Ian R. A. Mackenzie · Manuela Neumann · Eileen H. Bigio · Nigel J. Cairns · Irina Alafuzoff · Jillian Kril · Gabor G. Kovacs · Bernardino Ghetti · Glenda Halliday · Ida E. Holm · Paul G. Ince · Wouter Kamphorst · Tamas Revesz · Annemieke J. M. Rozemuller · Samir Kumar-Singh · Haruhiko Akiyama · Atik Baborie · Salvatore Spina · Dennis W. Dickson · John Q. Trojanowski · David M. A. Mann

Received: 20 October 2008 / Revised: 10 November 2008 / Accepted: 10 November 2008

Introduction

The neuropathology associated with the clinical entities frontotemporal dementia (FTD, behavioral variant FTD), progressive non-fluent aphasia (PNFA) and semantic dementia (SD), is heterogeneous with the common feature being a relatively selective degeneration of the frontal and temporal lobes (frontotemporal lobar degeneration, FTLD). As in other neurodegenerative conditions, most pathological subtypes of FTLD are characterized by specific kinds of intracellular protein inclusions. In the past few decades, the biochemical composition of many of these inclusion bodies has been determined. There is a growing trend to classify FTLD based on the presumed molecular defect, in the belief that this most closely reflects the underlying pathogenic process and because many of the eponymous and descriptively named syndromes of the past are now known to have imperfect clinicopathological correlation.

I. R. A. Mackenzie
Department of Pathology and Laboratory Medicine,
Vancouver General Hospital, University of British Columbia,
Vancouver, BC, Canada

M. Neumann
Institute of Neuropathology,
University Hospital of Zurich, Zurich, Switzerland

E. H. Bigio
Department of Pathology,
Northwestern University Feinberg School of Medicine,
Chicago, IL, USA

N. J. Cairns
Department of Pathology and Immunology,
Washington University School of Medicine,
St Louis, MO, USA

I. Alafuzoff
Section of Neuropathology,
Kuopio University, Kuopio, Finland

J. Kril
Department of Pathology,
The University of Sydney, Sydney, NSW, Australia

G. G. Kovacs
Institute of Neurology,
Medical University of Vienna, Vienna, Austria

B. Ghetti · S. Spina
Department of Pathology and Laboratory Medicine,
Indiana University School of Medicine,
Indianapolis, IN, USA

G. Halliday
Prince of Wales Medical Research Institute,
University of New South Wales, Sydney, NSW, Australia

I. E. Holm
Department of Pathology, Aalborg Hospital,
Aarhus University Hospital, Aalborg, Denmark

P. G. Ince
Neuropathology Group,
University of Sheffield Medical School, Sheffield, UK

W. Kamphorst · A. J. M. Rozemuller
Department of Pathology, Vrije University Medical Centre,
Amsterdam, The Netherlands

T. Revesz
Department of Molecular Neuroscience,
Queen Square, London, UK

S. Kumar-Singh
Department of Molecular Genetics,
University of Antwerp, Antwerp, Belgium

A comprehensive consensus paper on the neuropathologic diagnostic and nosologic criteria for FTLD was recently published in this journal [3]. These criteria incorporate several important recent advances in our understanding of the molecular genetics and pathology of FTLD; specifically, the discovery of several new FTLD-associated gene abnormalities and the identification of TDP-43 as the pathological protein in most tau-negative FTLD. The criteria employ a protein-based approach for the neuropathologic diagnosis and classification of FTLD; however, the nomenclature for individual conditions has not been revised to reflect this.

Specific problems with current nomenclature

Further advances in our understanding of the disease specificity and sensitivity of TDP-43 pathology have resulted in confusion around the use of the term "frontotemporal lobar degeneration with ubiquitinated inclusions" (FTLD-U). "FTLD-U" was originally developed for cases in which the characteristic inclusions are only visible with ubiquitin immunohistochemistry. It was anticipated that the ubiquitinated protein (or proteins) would eventually be identified and that this would allow more specific nomenclature and reclassification. Accordingly, when a small subset of cases was discovered to have inclusions that were also immunoreactive for class IV intermediate filaments, these were given a new designation (neuronal intermediate filament inclusion disease, NIFID) and removed from the FTLD-U category. However, when TDP-43 was recently identified as the pathological protein in most of the remaining cases of FTLD-U [2, 9], this convention was not immediately followed. FTLD-U has continued to be used with the assumption that all the remaining genetic and pathologic subtypes of FTLD-U are TDP-43 proteinopathies. However, recent studies have demonstrated that this is not the case; at least one familial subtype [the Danish kindred with autosomal dominant FTD linked to chromosome 3 (FTD-3), caused by a *CHMP2B* mutation] and a significant proportion of sporadic FTLD-U cases, do not have signatory pathological TDP-43 [4, 5, 7, 10]. Therefore, the group currently designated as FTLD-U appears to include several distinct entities, the largest of which (TDP-43-positive) no longer satisfies the original definition of the term.

A second area of uncertainty relates to the disease specificity of TDP-43 pathology. Although the initial reports suggested that pathological TDP-43 was specific for FTLD-U and ALS, several recent studies have found TDP-43-positive inclusions in a significant proportion of cases with other neurodegenerative conditions, such as Alzheimer's disease (AD), Lewy body disease and some primary tauopathies [1, 8, 12]. This TDP-43 pathology had not been recognized previously because ubiquitin immunohistochemistry does not distinguish it from the other coexisting (tau or α-synuclein) pathology. Although the concomitant TDP-43 pathology is usually restricted to limbic structures of the mesial temporal lobe, it sometimes extends into the neocortex and can closely resemble FTLD-U. It is currently not known if this represents a coincidental primary pathological process, which contributes to the clinical phenotype, or a secondary change of little pathogenic significance, occurring in susceptible neuronal populations. Furthermore, there are currently no neuropathologic criteria for FTLD-U that define the extent and anatomic distribution of pathology needed for the diagnosis. Therefore, pending further clinicopathological correlative studies, it is uncertain whether or not a diagnosis of FTLD-U should be made when TDP-43 pathology is found in conjunction with other neurodegenerative processes.

The following recommendations are meant to serve two purposes. First, to introduce a protein-based nomenclature for FTLD that is simple, consistent and transparent, and one that can easily accommodate future discoveries. Second, to modify existing terminology to address the specific issues related to FTLD-U and TDP-43 pathology, described above.

Recommendations

1. FTLD should be retained as the general terminology for pathological conditions that are commonly associated

H. Akiyama
Tokyo Institute of Psychiatry, Tokyo, Japan

A. Baborie
Neuropathology,
The Walton Centre for Neurology and Neurosurgery,
Liverpool, UK

D. W. Dickson
Neuropathology Laboratory,
Mayo Clinic College of Medicine, Jacksonville, FL, USA

J. Q. Trojanowski
Department of Pathology and Laboratory Medicine,
University of Pennsylvania School of Medicine,
Philadelphia, PA, USA

D. M. A. Mann
Clinical Neuroscience Research Group,
Greater Manchester Neurosciences Centre,
University of Manchester, Salford, UK

I. R. A. Mackenzie (✉)
Department of Pathology, Vancouver General Hospital,
855 West 12th Ave, Vancouver, BC V5Z 1M9, Canada
e-mail: ian.mackenzie@vch.ca

Table 1 Recommended nomenclature for frontotemporal lobar degenerations

Current terminology	Recommended new terminology	Major pathological subtypes[a]
tau-positive FTLD	FTLD-tau	PiD CBD PSP AGD MSTD Unclassifiable
tau-negative FTLD		
FTLD-U		
TDP-43-positive	FTLD-TDP	Type 1-4[b] Unclassifiable
TDP-43-negative	FTLD-UPS	aFTLD-U FTD-3
NIFID	FTLD-IF	
DLDH	FTLD-ni	
Other		
BIBD	BIBD	

aFTLD-U atypical frontotemporal lobar degeneration with ubiquitinated inclusions, *AGD* argyrophilic grain disease, *BIBD* basophilic inclusion body disease, *CBD* corticobasal degeneration, *DLDH* dementia lacking distinctive histopathology, *FTD-3* frontotemporal dementia linked to chromosome 3, *FTLD* frontotemporal lobar degeneration, *FTLD-U* FTLD with ubiquitinated inclusions, *IF* intermediate filament, *MSTD* multiple system tauopathy with dementia, *ni* no inclusions, *NIFID* neuronal intermediate filament inclusion disease, *PiD* Pick's disease, *PSP* progressive supranuclear palsy, *TDP* TDP-43, *UPS* ubiquitin proteosome system

[a] Indicates the characteristic pattern of pathology, not the clinical syndrome. Note that FTDP-17 is not listed as a pathological subtype because cases with different *MAPT* mutations do not have a consistent pattern of pathology. These cases would all be FTLD-tau, but further subtyping would vary

[b] Must specify which classification system is being used [6, 11]

with the clinical entities of FTD, PNFA and/or SD, and in which degeneration of the frontal and temporal lobes is a characteristic feature. It is recognized, however, that other anatomical regions (especially the parietal lobes and striatonigral system) may also be involved in some of these cases.

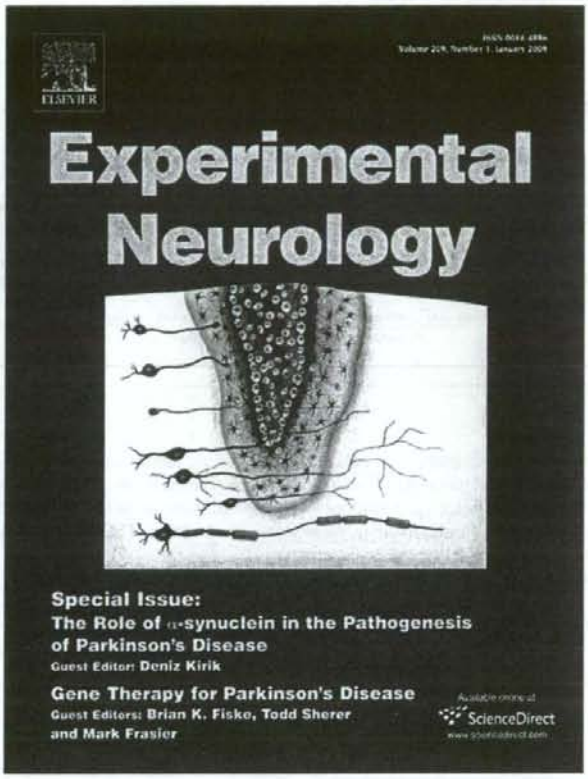
2. Major subdivisions should be designated by the protein abnormality that is presumed to be pathogenic or most characteristic of the condition (i.e. FTLD-protein) (Table 1).
3. When a new entity is discovered or when the molecular identity of the major pathological factor in an existing group is clarified, the appropriate term will be FTLD-pathological molecule.
4. Whenever possible, cases should be further sub-classified, using current terminology, to define the specific pattern of pathology [i.e. FTLD-tau (CBD) or FTLD-TDP (type 2)] (Table 1).
5. Cases with inclusions that can only be demonstrated with immunohistochemistry against proteins of the ubiquitin proteosome system (UPS), should be designated FTLD-UPS. This would include FTD-3 and the recently described cases of FTLD with ubiquitin-positive, TDP-43-negative inclusions [4, 5, 7, 10]. This designation recognizes that the TDP-43-negative inclusions may immunostain for UPS proteins other than ubiquitin, such as p62. This change should also avoid confusion with the previous terminology of FTLD-U.
6. Existing terms should be retained for rare causes of FTLD that have characteristic pathological features of unknown biochemistry, such as basophilic inclusion body disease (BIBD).
7. Cases of FTLD with no inclusions visible with special histochemical stains or the relevant immunohistochemistry should be designated FTLD-ni (no inclusions). This provides consistency in nomenclature and replaces the term "dementia lacking distinctive histopathology (DLDH), which many feel to be unsatisfactory because it suggests that pathologic changes are completely absent.
8. A diagnosis of FTLD–TDP should only be made in the presence of another (non-TDP-43) pathological process when the other pathology is considered too minor to have caused dementia (i.e., senile plaques or neurofibrillary tangles at densities below that required for the diagnosis of AD). When TDP-43 pathology is encountered in a case that fulfills neuropathologic criteria for some other neurodegenerative condition (such as AD), the presence and anatomical distribution of TDP-43 pathology should be indicated in a descriptive fashion [i.e. AD with limbic (or diffuse) TDP-43 pathology].

Summary

These recommendations provide a simple system of nomenclature that reflects our current understanding of the molecular pathology of FTLD and that can easily accommodate future discoveries. The terminology will allow neuropathologists to communicate their findings in a concise and unambiguous fashion. Terms that have become obsolete (i.e., FTLD-U) have been eliminated, while other traditional names for specific patterns of pathology within these broad protein-based categories can still be used without contradiction. This also provides a neuropathologic nosology that can be correlated with molecular genetic and clinical features. The next logical step will be to convene a meeting of international experts to update the clinical and pathological diagnostic criteria for FTLD and to develop an integrated classification scheme that reflects the many recent advances in the field.

References

1. Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW (2007) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol 61:435–445. doi:10.1002/ana.21154
2. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 351:602–611. doi:10.1016/j.bbrc.2006.10.093
3. Cairns NJ, Bigio EH, Mackenzie IRA, Neumann M, Lee VMY, Hatanpaa KJ, White CL, Schneider JA, Grinberg LT, Halliday G, Duyckaerts C, Lowe JS, Holm IE, Tolnay M, Okamoto K, Yokoo H, Murayama S, Woulfe J, Munoz DG, Dickson DW, Ince PG, Trojanowski JQ, Mann DMA (2007) Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol 114:2–22. doi:10.1007/s00401-007-0237-2
4. Holm IE, Englund E, Mackenzie IRA, Johannsen P, Isaacs A (2007) A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3 (FTD-3). J Neuropathol Exp Neurol 66:884–891. doi:10.1097/nen.0b013e3181567f02
5. Josephs KA, Lin WL, Ahmed Z, Stroh DA, Graff-Radford NR, Dickson DW (2008) Frontotemporal lobar degeneration with ubiquitin-positive, but TDP-43-negative inclusions. Acta Neuropathol 116:159–167. doi:10.1007/s00401-008-0397-8
6. Mackenzie IRA, Baborie A, Pickering-Brown S, du Pleissis D, Jaros E, Perry RH, Neary D, Snowden JS, Mann DMA (2006) Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. Acta Neuropathol 112:539–549. doi:10.1007/s00401-006-0138-9
7. Mackenzie IRA, Foti D, Woulfe J, Hurwitz TA (2008) Atypical frontotemporal lobar degeneration with ubiquitin-positive, TDP-43-negative neuronal inclusions. Brain 131:1282–1293. doi:10.1093/brain/awn061
8. Nakashima-Yasuda H, Uryu K, Robinson J, Xie SX, Jurtig H, Duda JE, Arnold SE, Siderowf A, Grossman M, Leverenz JB, Woltjer R, Lopez OL, Hamilton R, Tsuang DW, Galaska D, Masliah E, Kaye J, Clark CM, Montine TJ, Lee VMY, Trojanowski JQ (2007) Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol 114:221–229. doi:10.1007/s00401-007-0261-2
9. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McClusky LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VMY (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314:130–133. doi:10.1126/science.1134108
10. Roeber S, Mackenzie IR, Kretzschmar HA, Neumann M (2008) TDP-43-negative FTLD-U is a significant new clinico-pathological subtype of FTLD. Acta Neuropathol 116:147–157. doi:10.1007/s00401-008-0395-x
11. Sampathu DM, Neumann M, Kwong LK, Chou TT, Micsenyi M, Truax A, Bruce J, Grossman M, Trojanowski JQ, Lee VMY (2006) Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. Am J Pathol 169:1343–1352. doi:10.2353/ajpath.2006.060438
12. Uryu K, Nakashima-Yasuda H, Forman MS, Kwong LK, Clark CM, Grossman M, Miller BL, Kretzschmar HA, Lee VM, Trojanowski JQ, Neumann M (2008) Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. J Neuropathol Exp Neurol 67:555–564. doi:10.1097/NEN.0b013e31817713b5



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Short Communication

Apoptosis of primary sensory neurons in GD1b-induced sensory ataxic neuropathy

Kazuo Takada [a], Jun Shimizu [b], Susumu Kusunoki [a,*]

[a] Department of Neurology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
[b] Department of Neurology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Received 22 May 2007; revised 4 September 2007; accepted 10 September 2007
Available online 21 September 2007

Abstract

Experimental autoimmune sensory ataxic neuropathy was induced in three of six rabbits sensitized with GD1b ganglioside (GD1b-SAN). TUNEL assay was performed on sections of dorsal root ganglia in the cauda equina. The results showed the presence of TUNEL-positive neurons in all three rabbits affected with GD1b-SAN. In contrast, no such neurons were observed in any of the sections from the unaffected rabbits that had been inoculated with GD1b, rabbits inoculated with adjuvant alone or those without inoculation. These data support that an apoptotic mechanism is involved in the pathogenesis of GD1b-SAN.

Keywords: Ganglioside; Neuropathy; Guillain–Barré syndrome; Autoimmunity; Antibody; Apoptosis; Sensory neuron; Dorsal root ganglion

Introduction

Antiganglioside antibodies are frequently present in sera from patients with autoimmune neuropathies, such as Guillain–Barré syndrome and IgM paraproteinemic neuropathy (Kusunoki, 2000; Willison and Yuki, 2002). Immunohistochemical studies have demonstrated that some gangliosides exhibit a unique localization in the nervous system (Kusunoki et al., 1993; Chiba et al., 1993; Kaida et al., 2003). The antibodies to these gangliosides may therefore be associated with unique clinical features by binding to regions in which the target antigen gangliosides are localized.

Immunohistochemistry using a monoclonal antibody against GD1b ganglioside has shown that GD1b is densely localized in the large neurons in the dorsal root ganglia (DRGs) of rabbits as well as humans (Kusunoki et al., 1993, 1996). These large neurons are known to mediate proprioception. IgM M-proteins that recognize a disialosyl residue of GD1b are specifically present in sera from patients with sensory ataxic neuropathy (Willison et al., 2001). We have previously reported that sensitization of rabbits with GD1b induces sensory ataxic neuropathy (GD1b-SAN) (Kusunoki et al., 1996), in which IgG antibodies monospecific to GD1b is essential to the pathogenesis of the disease (Kusunoki et al., 1999a). GD1b-SAN is the first established animal model of autoimmune neuropathy mediated by antiganglioside antibodies. Passive transfer of anti-GD1b antisera from rabbits affected with GD1b-SAN-induced degeneration of rabbit sensory neurons, indicating that anti-GD1b antibody is directly involved in the pathogenesis of GD1b-SAN (Kusunoki et al., 1999b). The precise mechanism by which the anti-GD1b antibody causes the disease remains to be elucidated.

Pathological investigation of GD1b-SAN demonstrated that there was no lymphocytic infiltration in the affected regions. Degeneration of the axons was evident in the dorsal root and dorsal column with macrophage infiltration (Kusunoki et al., 1996). In contrast, in spite of the looseness of the blood–nerve barrier in the DRG, there was no significant finding in the DRG except for few Nageotte nodules (Kusunoki et al., 1996). This prompted us to investigate the possibility that the anti-GD1b antibody induces apoptosis of the large sensory neurons.

* Corresponding author. Fax: +81 72 368 4846.
E-mail address: kusunoki-tky@umin.ac.jp (S. Kusunoki).

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doi:10.1016/j.expneurol.2007.09.010

Through the use of terminal deoxynucleotidyl tranferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) assay, we found evidence of apoptosis in the DRGs from GD1b-SAN-affected rabbits.

Materials and methods

Immunization with GD1b was performed as described previously (Kusunoki et al., 1996). Six rabbits were immunized

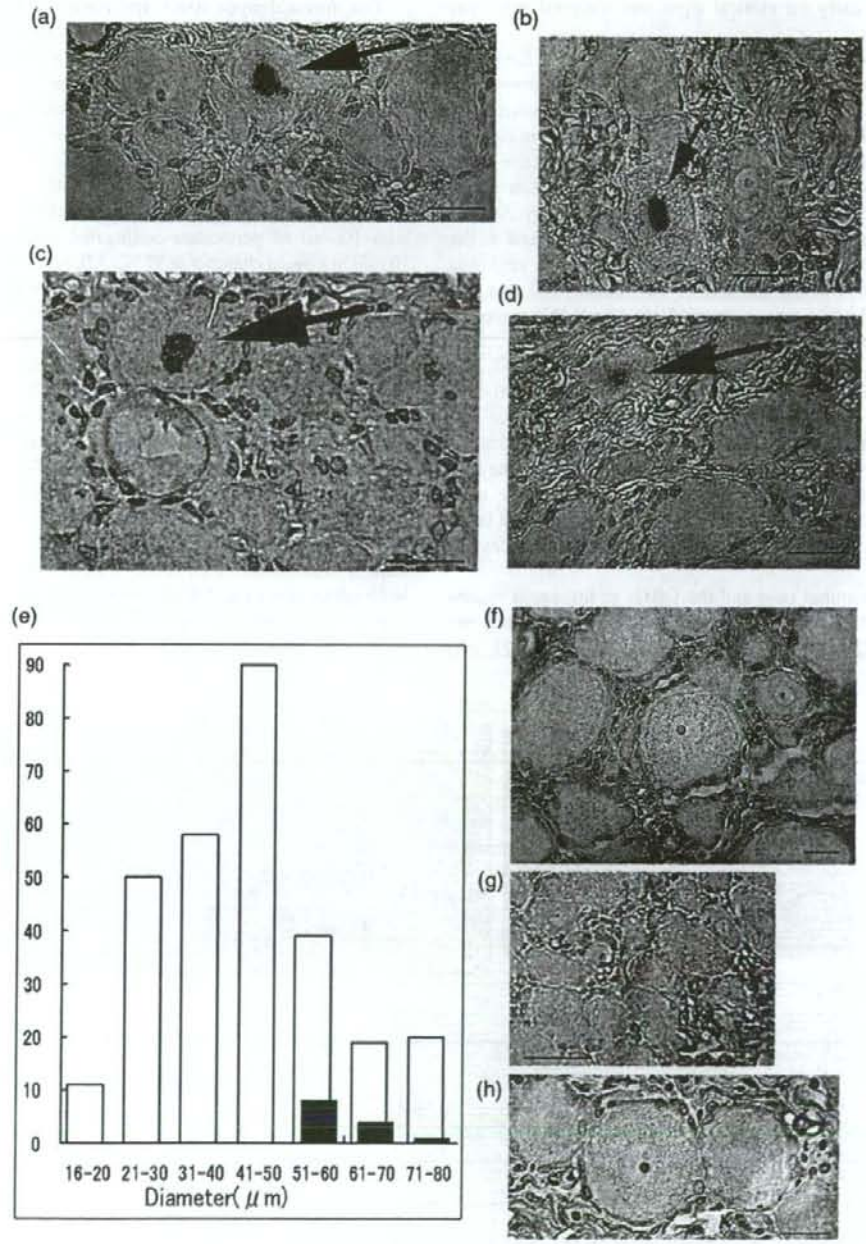

Fig. 1. (a–d) TUNEL assay of sections from rabbits affected with GD1b-SAN. Primary sensory neurons containing nucleotides that react positively (arrows). Scale bar = 50 μm. (e) The histogram of the diameters of TUNEL-positive and TUNEL-negative neurons. The TUNEL-positive neurons were of large ones. (f–h) TUNEL assay of sections from unaffected rabbits inoculated with GD1b (f), inoculated with adjuvant alone (g) and without any inoculation (h). No TUNEL-positive cells were observed. Scale bar = 50 μm.

with GD1b, and two were given the same inoculum without GD1b. Two rabbits without inoculation were also kept in the same room of the animal center. Serum samples were taken by ear vein puncture at 1- or 2-week intervals. The rabbits were checked daily for clinical signs and weighed twice per week.

Serum anti-GD1b antibodies were investigated by the use of enzyme-linked immunosorbent assay, as described previously (Kusunoki et al., 1996). Microtiter wells were coated with 200 ng of GD1b, with an uncoated well serving as the control. Peroxidase-conjugated antibody to rabbit IgM (mu-chain specific; Cappel, West Chester, PA; diluted 1:400) or rabbit IgG (gamma-chain specific; Southern Biotechnology Associates Inc., Birmingham, USA; diluted 1:2000) was used as the secondary antibody. The optical density (OD; 492 nm) was corrected by subtracting the OD of the control well that had been processed in the same manner. A reaction with a corrected OD of more than 0.1 was considered positive.

The rabbits affected with GD1b-SAN were sacrificed 2 or 3 days after the neurological onset (namely, 35, 42 and 90 days after the first inoculation, respectively). The rabbits that had been immunized with GD1b but did not exhibit any neurological problems were sacrificed 121, 136 and 156 days after the first inoculation. The rabbits inoculated with adjuvant alone were sacrificed on day 35 and 42 after the first inoculation and those without any inoculation were sacrificed after 14 and 21 days of observation.

The lumbar spinal cord and the DRGs in the cauda equina were removed from all of the rabbits except for the two unaffected GD1b-sensitized rabbits (sacrificed on 121 and 136 days after first inoculation). Specimens were fixed either in 4% formaldehyde in phosphate-buffered saline for 48 h or in 2.5% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M sodium cacodylate (half Karnovsky solution) for 12 h.

The formaldehyde-fixed specimens were embedded in paraffin and serial sections, 10 μm in thickness, were prepared. TUNEL assay was performed using a kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions. Briefly, the sections underwent protein digestion at 37 °C for 5 min, after deparaffinization and hydration. After washing, they were incubated with 50 μl of TdT reaction solution for 10 min in a moist chamber at 37 °C. After inactivation of intrinsic peroxidase, sections were incubated with 100 μl of peroxidase-conjugated antibody solution for 10 min in a moist chamber at 37 °C. After removing the antibody solutions, they were incubated with 100 μl of diaminobenzidine solution for 5 min at room temperature. After washing, sections were dehydrated and covered with a cover glass. The sections of DRGs in the cauda equina obtained from each of the three rabbits affected with GD1b-SAN, each of the two adjuvant controls, one unaffected rabbit sensitized with GD1b, and two rabbits without inoculation were examined with TUNEL assay. Seventy sections from each rabbit were examined. In the DRGs from the affected rabbits, the diameters of the TUNEL-positive and TUNEL-negative neurons containing a nucleus were measured. Immunohistochemistry with anti-caspase 3 antibody also was performed on deparaffinized sections as described by Gown and Willingham (Gown and Willingham, 2002). Mouse monoclonal anti-caspase 3 (3G2, 1:40 diluted, Abcam, Cambridge, UK) was used as the primary antibody, and peroxidase-conjugated goat

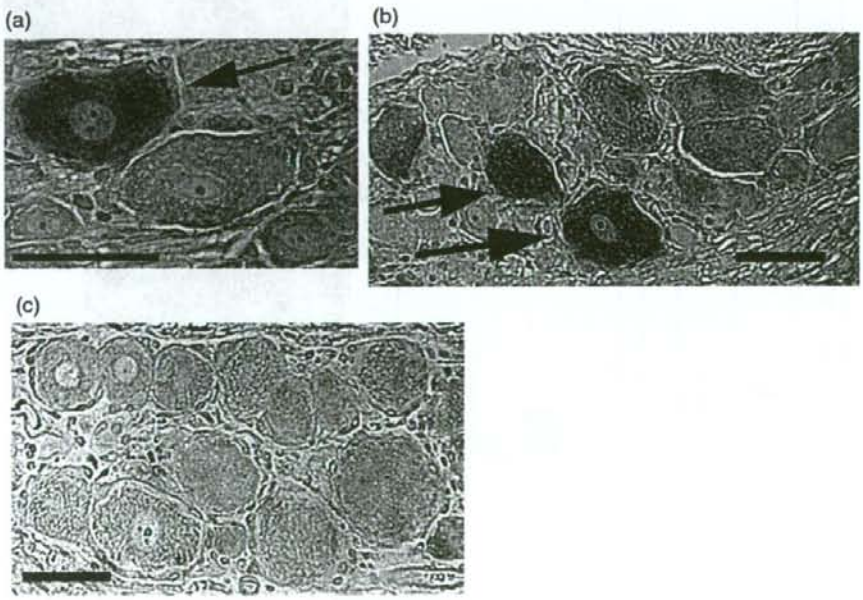

Fig. 2. Immunohistochemistry using anti-caspase 3 antibody. (a and b) Some DRG neurons from rabbits affected with GD1b-SAN were immunostained with anti-caspase 3 antibody (arrows). (c) No such staining was observed in DRG from a rabbit inoculated with adjuvant alone. Scale bar = 50 μm.

anti-mouse IgG (1:250 diluted, MP Biomedicals, Ohio, USA) was the secondary antibody.

The Karnovsky-fixed specimens were postfixed in 2% osmium tetroxide for 2 h, dehydrated with ethanol and embedded in epoxy resin. Semi-thin sections, 1.0 μm in thickness, were cut from the Epon block and were stained with toluidine blue.

The experiments involving animals were approved by the local ethics committee in Kinki University.

Results

Anti-GD1b IgM antibody was detected 2 weeks after the first inoculation and reached a maximum at 4 weeks. IgG anti-GD1b antibody was elevated later, after the elevation of IgM.

Three of the six rabbits immunized with GD1b developed SAN 33, 40 and 88 days after the first inoculation, respectively.

In the sections stained with toluidine blue, axonal degeneration of the dorsal column of the spinal cord was evident, as described previously.

A few TUNEL-positive cells were observed in the formaldehyde-fixed sections from all three rabbits affected with GD1b-induced SAN (Figs. 1a–d). Two sections were mounted on each glass slide, in which approximately one TUNEL-positive cell was observed. In contrast, no TUNEL-positive cells were observed in any section from the unaffected rabbits that had been immunized with GD1b (Fig. 1f), the rabbits inoculated with adjuvant alone (Fig. 1g) or the rabbits without inoculation (Fig. 1h).

Of the 300 neurons of DRGs from the affected rabbits, 13 were TUNEL-positive and 287 were TUNEL-negative. The histogram was shown in Fig. 1e.

A few neurons per section of DRG from the affected rabbits were immunostained with anti-caspase 3 antibody, whereas no such staining was seen in DRG from control rabbits (Fig. 2).

Discussion

Anti-GD1b antibodies were detected in all six rabbits sensitized with GD1b. Three of those six rabbits developed SAN. This finding is compatible with the previous results that about half of the rabbits sensitized with GD1b developed SAN (Kusunoki et al., 1996, 1999a).

We previously reported that downregulation of trkC occurs in the dorsal root ganglia from rabbits in the acute phase of GD1b-SAN (Hitoshi et al., 1999). TrkC serves as a receptor for neurotrophin-3 (NT3). It is known that the large primary sensory neurons in the dorsal root ganglia, which mediate proprioception, depend mainly on neurotrophin-3-mediated trkC signaling. It has been reported that mice defective for trkC exhibit abnormal movement due to lack of proprioception (Klein et al., 1994). Our above result of trkC downregulation therefore suggests that anti-GD1b antibody-mediated trkC downregulation and subsequent apoptosis of the large neurons of DRG contribute to the pathogenesis of GD1b-SAN.

The present investigation provides clear evidence that an apoptotic mechanism is involved in the pathogenesis of GD1b-SAN. Positive immunostaining of some DRG neurons from affected rabbits with anti-caspase 3 antibody also indicates the involvement of an apoptotic mechanism in GD1b-SAN. Although the number of neurons with apoptotic changes appears to be small, approximately 4% of neurons were TUNEL-positive, indicating that quite a few sensory neurons could be affected in each animal. An apoptotic neuron should not remain *in situ* for a long time but would soon disappear. Therefore, we were not able to identify many apoptotic neurons at the same time in the pathological specimens.

Gangliosides are known to form microdomains called lipid rafts (Simons and Toomre, 2000). Within the rafts, gangliosides are believed to interact with important transmembrane receptors or signal transducers (Kasahara et al., 2000). Treatment of rat cerebellar cultures with a monoclonal anti-ganglioside GD3 antibody induced the activation of the Src family kinase Lyn and rapid tyrosine phosphorylation of some proteins (Kasahara et al., 1997). Thus, antiganglioside antibodies may alter the function of neurons through binding to target gangliosides in the raft. The mechanism(s) by which antibody binding causes apoptosis and whether the downregulation of trkC is involved in the process need to be clarified in future investigations.

Neuropathy with IgM M-protein binding to a disialosyl residue of several gangliosides, including GD1b, GT1b and GQ1b, is a human counterpart of GD1b-SAN (Willison et al., 2001). There has been one reported autopsy case of this kind of neuropathy, demonstrating a reduction in the number of sensory neurons in the DRG and pallor of the dorsal column in the spinal cord (Obi et al., 1999). In addition to the IgM paraproteinemic neuropathy, GBS with a monospecific anti-GD1b IgG antibody has been associated with ataxia due to disturbance in deep sensation (Wicklein et al., 1997). It has been reported that human IgM monoclonal antibody recognizing GD2, GD1b, GT1b and GQ1b resulted in death of rat dorsal root ganglion neurons (Ohsawa et al., 1993). However, the precise mechanism was not elucidated. Our present findings strongly suggest that apoptosis of the large primary sensory neurons contributes to the pathogenesis of human neuropathies with antibodies recognizing gangliosides with disialosyl residue, in particular GD1b.

Acknowledgments

This work was supported in part by Grants-in Aid for Scientific Research (18390264) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by a Health Sciences Research Grant on Psychiatric and Neurological Diseases and Mental Health and a Research Grant for Neuroimmunological Diseases from the Ministry of Health, Labour and Welfare of Japan.

References

Chiba, A., Kusunoki, S., Obata, H., Machinami, R., Kanazawa, I., 1993. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain–Barre syndrome: clinical and immunohistochemical studies. Neurology 43, 1911–1917.

Gown, A.M., Willingham, M.C., 2002. Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. J. Histochem. Cytochem. 50, 449–454.

Hitoshi, S., Kusunoki, S., Murayama, S., Tsuji, S., Kanazawa, I., 1999. Rabbit experimental sensory ataxic neuropathy: anti-GD1b antibody-mediated trkC downregulation of dorsal root ganglia neurons. Neurosci. Lett. 260, 157–160.

Kaida, K., Kusunoki, S., Kamakura, K., Motoyoshi, K., Kanazawa, I., 2003. GalNAc-GD1a in human peripheral nerve: target sites of anti-ganglioside antibody. Neurology 61, 465–470.

Kasahara, K., Watanabe, Y., Yamamoto, T., Sanai, Y., 1997. Association of Src family tyrosine kinase Lyn with ganglioside GD3 in rat brain. Possible regulation of Lyn by glycosphingolipid in caveolae-like domains. J. Biol. Chem. 272, 29947–29953.

Kasahara, K., Watanabe, K., Takeuchi, K., Kaneko, H., Oohira, A., Yamamoto, T., Sanai, Y., 2000. Involvement of gangliosides in glycosylphosphatidylinositol-anchored neuronal cell adhesion molecule TAG-1 signaling in lipid rafts. J. Biol. Chem. 275, 34701–34709.

Klein, R., Silos-Santiago, I., Smeyne, R.J., Lira, S.A., Brambilla, R., Bryant, S., Zhang, L., Snider, W.D., Barbacid, M., 1994. Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. Nature 368, 249–251.

Kusunoki, S., 2000. Antiglycolipid antibodies in Guillain–Barre syndrome and autoimmune neuropathies. Am. J. Med. Sci. 319, 234–239.

Kusunoki, S., Chiba, A., Tai, T., Kanazawa, I., 1993. Localization of GM1 and GD1b antigens in the human peripheral nervous system. Muscle Nerve 16, 752–756.

Kusunoki, S., Shimizu, J., Chiba, A., Ugawa, Y., Hitoshi, S., Kanazawa, I., 1996. Experimental sensory neuropathy induced by sensitization with ganglioside GD1b. Ann. Neurol. 39, 424–431.

Kusunoki, S., Hitoshi, S., Kaida, K., Murayama, S., Kanazawa, I., 1999a. Degeneration of rabbit sensory neurons induced by passive transfer of anti-GD1b antiserum. Neurosci. Lett. 273, 33–36.

Kusunoki, S., Hitoshi, S., Kaida, K., Arita, M., Kanazawa, I., 1999b. Monospecific anti-GD1b IgG is required to induce rabbit ataxic neuropathy. Ann. Neurol. 45, 400–403.

Obi, T., Murakami, T., Takatsu, M., Kusunoki, S., Serizawa, M., Mizoguchi, K., Koike, R., Nishimura, Y., 1999. Clinicopathological study of an autopsy case with sensory-dominant polyradiculoneuropathy with antiganglioside antibodies. Muscle Nerve 22, 1426–1431.

Ohsawa, T., Miyatake, T., Yuki, N., 1993. Anti-B-series ganglioside-recognizing autoantibodies in an acute sensory neuropathy patient cause cell death of rat dorsal root ganglion neurons. Neurosci. Lett. 157, 167–170.

Simons, K., Toomre, D., 2000. Lipid rafts and signal transduction. Nat. Rev., Mol. Cell Biol. 1, 31–39.

Wicklein, E.M., Pfeiffer, G., Yuki, N., Hartard, C., Kunze, K., 1997. Prominent sensory ataxia in Guillain–Barre syndrome associated with IgG anti-GD1b antibody. J. Neurol. Sci. 151, 227–229.

Willison, H.J., Yuki, N., 2002. Peripheral neuropathies and anti-glycolipid antibodies. Brain 125, 2591–2625.

Willison, H.J., O'Leary, C.P., Veitch, J., Blumhardt, L.D., Busby, M., Donaghy, M., Fuhr, P., Ford, H., Hahn, A., Renaud, S., Katifi, H.A., Ponsford, S., Reuber, M., Steck, A., Sutton, I., Schady, W., Thomas, P.K., Thompson, A.J., Vallat, J.M., Winer, J., 2001. The clinical and laboratory features of chronic sensory ataxic neuropathy with anti-disialosyl IgM antibodies. Brain 124, 1968–1977.

MRI上，脳幹部にring enhancementを呈した急性型神経Behçet病の長期追跡剖検例

崎山快夫　齋藤尚大　齊藤祐子
吉野正俊　村山繁雄

神経内科
Reprinted from NEUROLOGICAL MEDICINE
Vol. 68 No. 6 June 2008
科学評論社

原著

MRI上，脳幹部にring enhancementを呈した急性型神経Behçet病の長期追跡剖検例*

崎山快夫**　齋藤尚大***[1]　齊藤祐子****
吉野正俊***　村山繁雄**

Key Words：neuro-Behçet disease, acute type, autopsy, long-term follow-up, ring enhancement

はじめに

Behçet病における中枢神経病変は上矢状静脈血栓症など血管病変に起因するものと(約20%)，脳実質に起因するもの(約80%)とに大別され，後者を狭義の神経Behçet病と呼ぶことが多い[1]．神経Behçet病は，その臨床症状と経過により，急性増悪と寛解の経過をとる急性型と，進行性の経過をとる慢性進行型に大別することができる[2]．われわれは，神経Behçet病の急性期病巣として稀な脳幹部のring enhancementを認めた急性型神経Behçet病報告例[3]を長期追跡し，剖検を得たので報告する．また，後の経過と合わせて総合的に評価するため，既報告の内容と一部重複しているが，詳細に再掲した．

症　例

患者：2005年，死亡時80歳，男性．
主訴：歩行障害．
家族歴：特記事項はない．
生活歴：喫煙歴は15本/日，飲酒歴は機会飲酒．
既往歴：1994年，69歳時に胆石を指摘され内服加療．
現病歴：1990年，65歳頃から口腔内や舌に小潰瘍が頻発．67歳頃から両下肢に有痛性の結節性紅斑様皮疹が頻発，陰嚢に潰瘍ができるようになった．69歳時，頭痛，発熱をしばしば認めたが市販薬内服で経過を観察していた．1995年，70歳時に頭痛と歩行時のふらつきを認めるようになり，歩行中に壁にぶつかったり，転倒したりするようになった．また，物静かな性格から大声で多弁となった．1カ月後に構音障害も出現し，近医より紹介され入院した．
入院時身体所見：身長154cm，体重51kg．体温37.2℃，脈拍60/分・整，血圧120/68mmHg，呼吸数14/分．顔面に挫瘡様皮疹，舌左縁に径5mmの潰瘍，上肢に毛嚢炎，両下肢に有痛性結節性紅斑を数個，陰嚢正中に痂皮を認めた．針反応では，膿疱は認めなかったが発赤が生じ，皮膚の被刺激性の亢進が認められた．

* A case of neuro-Behçet disease presenting with ring enhancement in the pontine base by MR imaging. A long-term follow-up study with autopsy.(Accepted April 4，2008).
** Yoshio SAKIYAMA, M.D. & Shigeo MURAYAMA, M.D., Ph.D.: 東京都老人総合研究所老年病ゲノム・ブレインバンク〔〒173-0015 東京都板橋区栄町35-2〕; Department of Neuropathology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0015, Japan.
*** Takahiro SAITO, M.D., Masatoshi YOSHINO, M.D. & **** Yuko SAITO, M.D., Ph.D.: 東京都老人医療センター神経内科，**** 剖検病理科; Departments of Neurology and **** Anatomical Pathology, Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan.
[1] 現　東京都立豊島病院神経科

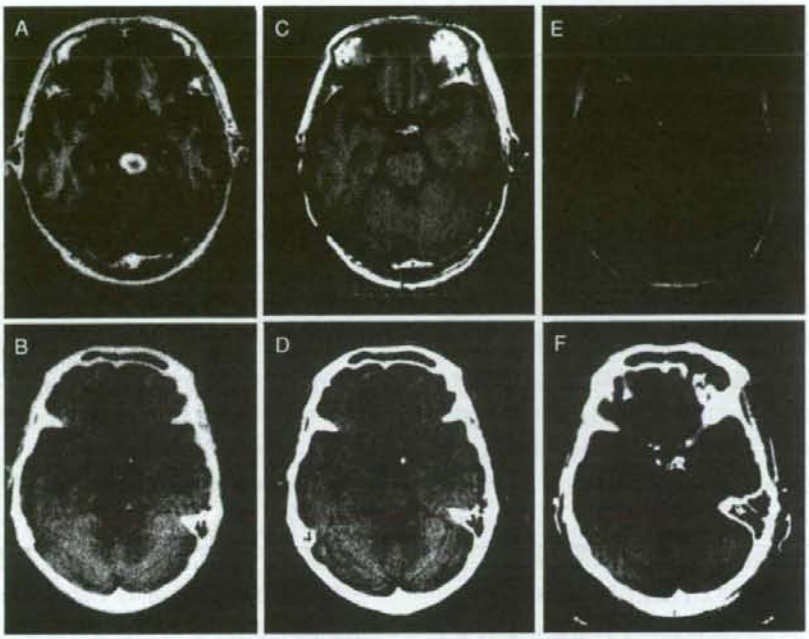

図1　本例の画像所見（上段：頭部MRI T1強調画像，AはGadolonium造影撮影．下段：頭部単純CT．A，Bは既報告（百瀬ら[3]より筆頭著者の許可を得て抜粋）
発症時の頭部MRI T1強調画像Gadolinium造影撮影(A)では，橋底部にring enhancementを伴う病変を認める．頭部CT(B)では，ring enhancementの中心に相当する部位に高吸収域を認め周囲に低吸収域を認めている．神経Behçet病発症2年後のMRI(C)，4年後のCT(D)では，発症時と比較して病巣は縮小している．発症9年後の頭部単純MRI(E)，CT(F)では，明らかな萎縮の進行は認めない．

入院時神経学的所見：多幸的児戯的性格変化，改訂型長谷川式簡易認知症スケール24/30，WAIS-R IQ 73点（VIQ 77，PIQ 74，落ち着きがなく途中で眠ってしまう），左視力低下（右1.0，左0.2）があり，眼底所見は左黄斑部下方血管の白線化と血管周囲の白鞘化を伴う変性を指摘され，網脈絡炎の既往を示唆する所見であった．右軽度不全麻痺を認め，四肢深部腱反射，下顎反射は亢進，右Babinski徴候を認めた．失調は明らかではなく，軽度の右麻痺による歩行障害と考えられた．

検査所見：血液検査で，白血球11,500/mm³（正常値4,800～7,500），CRP 4.3mg/dl（正常値0.3以下），血沈27mm/時（正常値10以下），便潜血陽性．脳脊髄液細胞数38/mm³（L：N＝88.3：12.7）（正常値0～3），蛋白41.6mg/dl（正常値20～45），糖53mg/dl（正常値50～100），IL-6 119pg/ml（正常値4.5以下[4]）のほか，血算・生化学・検尿所見に異常を認めなかった．HLA-B51は陰性であった．

画像所見：初診時の画像所見では，MRI T1強調造影画像で左橋底部にring enhancementを認め（図1-A），さらに左大脳脚に造影効果を認めた．T2強調画像では，同レベルの橋は広範な高信号域を示し，中央には低信号域が認められた．また，左後頭葉にT1低信号，T2高信号域を認めたが，これは造影されなかった．頭部単純CTでは左橋底部から視床にかけて内部に点状の高信号域を伴う低信号域が広がっていた（図1-B）．

入院後経過：自然経過で症状が軽快し，MRIにおける造影効果も減弱し，髄液細胞数も8/mm³と減少していたが，1カ月後に神経症状，粘膜皮膚所見の再増悪が認められ，髄液細胞数も810.6/mm³（L：N＝67.6：743）と再上昇を認めたため，治療を開始した．ステロイドパルス療法（メチルプレドニゾロン1,000mg/日3日間）の後，プレドニゾロン60mg/日内服開始したところ，臨床症状，髄液所見の著明な改善を認めた．その後，プレドニゾロン漸減し20mg/dayとして多幸性や興奮性も軽快したため退院した．

退院後経過：退院後外来で経過観察され，軽度の口内炎を認めたが，神経症状に著変はなく，多幸的で児戯的な性格は退院時から著変を認めなかった．72歳時に胃癌を指摘され，噴門側胃切除術および胆嚢摘出術を施行された．このとき施行したMRI(図 1-C)では，T1強調画像で造影効果を認めた部位は大幅に縮小，左橋底部は萎縮を示したほか，新しい病変は認めなかった．退院後食事がのどにつかえるという訴えを繰り返し，術後吻合部狭窄を認め内視鏡的拡張術をしばしば入院で施行，73歳時に再食道空腸吻合術を施行した．この頃にプレドニゾロン漸減，中止となった．その後も神経症状に著変なく経過した．1999年，74歳時にCTを施行されている(図 1-D)が，萎縮の進行は認めなかった．2004年，79歳時に食欲低下で短期入院を繰り返し，頭部MRI(図 1-E)，CT(図 1-F)を施行されているが，急性期の所見は認めず，萎縮の進行も明らかではなかった．タバコを吸うため勝手に院外に出てしまうことを繰り返し，入院継続は困難であった．80歳時の2005年7月，るいそう，衰弱が著しく入院．対症療法に反応せず第24病日に死亡した．ご遺族の同意を得て死後8時間で剖検．

全身病理所見：高度の器質化肺炎を認め，死因と考えられた．

神経病理所見：脳重1,095g．肉眼的に，左吻側橋底部背外側から正中にかけて線状の褐色沈着を伴う萎縮病巣を認めた(図 2)．この病変は連続して黒質網状層・大脳脚腹側・視床下部・淡蒼球内節に広がっており，左後頭極にも萎縮病巣を認めた．組織学的に，橋の病変は髄鞘が完全に消失しているかたちをとり，抗リン酸化ニューロフィラメント抗体(SMI31)免疫染色で軸索を，Klüver-Barrera 染色で髄鞘を評価したところ，軸索よりも髄鞘の消失の方がより範囲が広く脱髄の要素をもつと考えられた(図 3)．また，辺縁が不鮮明であり二次性の脱髄所見と考えられた．この病変の中心部(図 4)では中心染色質融解を示す神経細胞を認め，周辺の血管周囲にはヘモジデリン貪食マクロファージを多数認めた．リンパ球のカッフィングを少数認め，これらは抗CD45RO(UCHL1)抗体陽性であった．リンパ球浸潤は髄膜，後根神経節や脳脊髄の小血管周囲

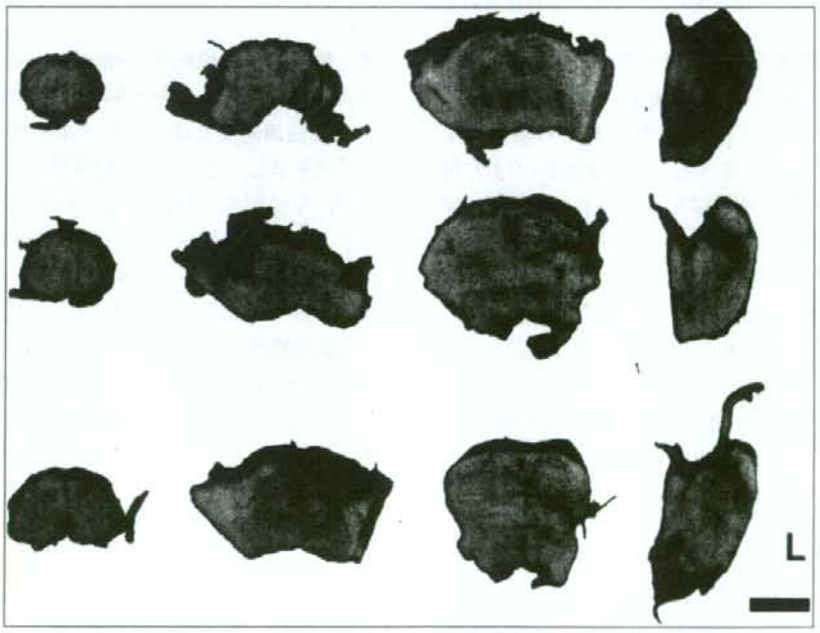

図 2　症例のホルマリン固定後脳幹軸状断
吻側橋底部正中から左にかけて線状の瘢痕を認め，この病変は左黒質網状層・大脳脚腹側に続いている．

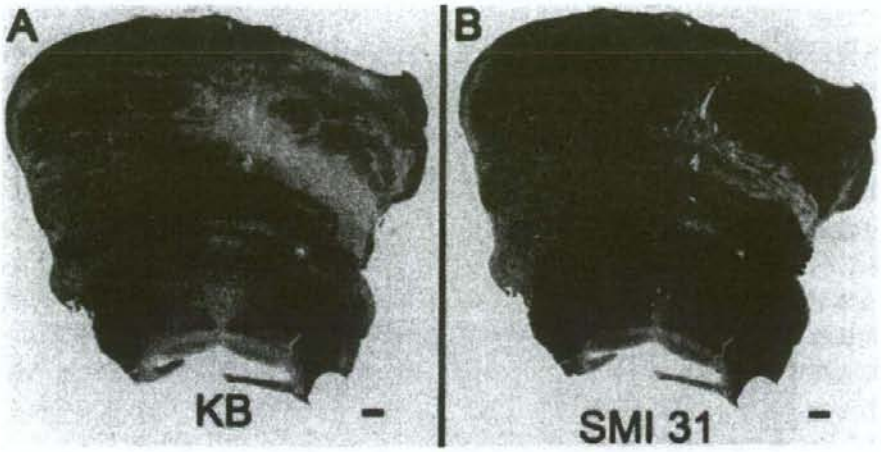

図3　発症時輪状造影効果を認めた橋のルーペ像(A：Klüver-Barrera染色，B：抗リン酸化ニューロフィラメント抗体(SMI31)免疫染色，Bar＝1 mm)
軸索の消失している範囲(B)に比較して髄鞘の失われている範囲(A)の方が広く周辺部は脱髄と考えられる．また，脱髄巣の辺縁は不明瞭で，二次性脱髄が示唆される．

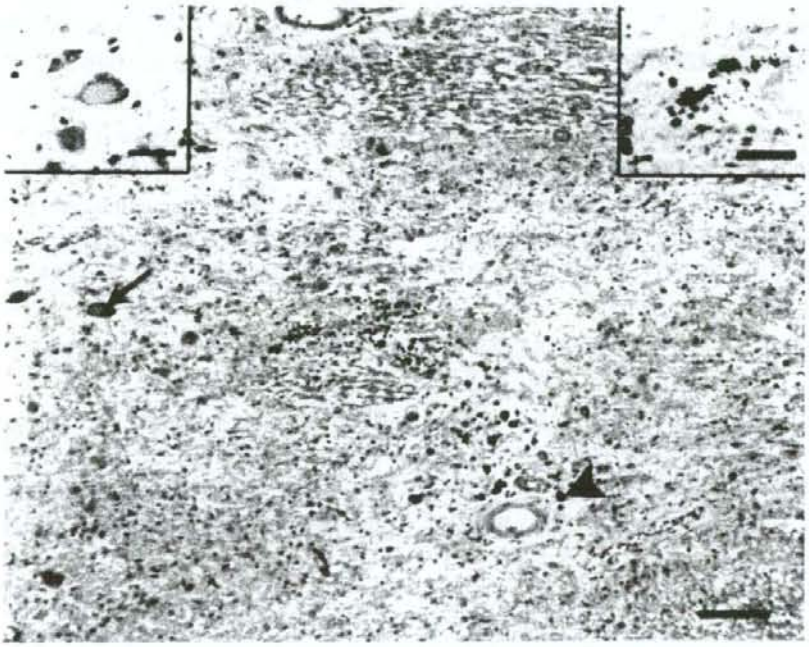

図4　橋底部病変周辺の小血管を含む拡大像(中央：HE染色，Bar＝100μm×10．左上インデント：Klüver-Barrera染色，Bar＝25μm×40．右上インデント：ベルリン青染色，Bar＝25μm×40)
小血管周囲に多数のヘモジデリンの沈着(矢尻)を認め，鉄染色(右上)で陽性となる．また，リンパ球を少数認める．病変の辺縁部に残存する神経細胞には中心染色質融解(矢印，左上)を認める．

にも少数ながら局所性に散在を認めた．

考　察

本症例の経過をまとめると，口腔粘膜のアフタ性潰瘍で初発し，結節性紅斑，陰部潰瘍が出現し，網脈絡膜炎の既往を示唆する所見を確認

されており，厚生労働省の基準ではBehçet病の臨床診断基準の4主症状を満たす完全型である．国際的にはInternational Study Groupから再発性の口腔内アフタを重視し，他を副症状とする診断基準[1)5)]が提唱されているが，これを用いても，Behçet病の診断基準を満たす症例と考えられる．経過5年で神経Behçet病を発症し，脳幹に歩行障害・精神症状が前景に立ち，脳幹を中心とする病変を認めているが，これについても典型的な経過と考えられる[4)6)]．

神経Behçet病の分類は症状と局在からの分類があり[7)~9)10)]，大まかに髄膜炎型と脳幹型に分類することができるが，それによれば，本例は脳幹型に分類される．精神症状としても，神経Behçet病の脳幹型に多い症状として人格退行[6)11)]があげられており，脳幹型に典型的な症状であったといえる．しかし，その予後との関連から経過によって急性型，慢性型に分類する方が実用的であるとの考えが一般的となっており[2)4)12)13)]，本例は70歳のエピソードの後10年間にわたり再発を認めず，画像上も慢性型に認めるような進行性の萎縮は示さず，急性型の寛解後長期経過例に分類される．

画像所見について既報[3)]では，ring enhancementの内部は出血を示しており，外郭は脳血液関門の障害部分を表現したものではないかと解釈されている[3)]．今回この部位に対応する神経病理所見としては，炎症に伴う二次性の脱髄の要素をもつ萎縮性の瘢痕と，その内部における小血管周囲を中心としたヘモジデリン沈着が認められ，既報告の背景病理を確認することができた．神経Behçet病の発症には一般に血管病変と密接に関連し[13)]，その組織学的特徴は脳実質炎症においては最小動脈から毛細血管・小静脈に至る小血管周囲のリンパ球浸潤であり，不完全な脱髄所見の様相を呈するとされている[14)]．これらのことを合わせると，神経Behçet病におけるMRIの造影効果は小血管周囲への炎症細胞浸潤によるものであるが，静脈を巻き込んだ炎症であるために出血を伴いやすく，病巣の内部に出血を伴った場合に中心の造影効果が失われring enhancementを示すと考えられる．Ring enhancementを伴う神経Behçet病の報告は稀で，Imotoらは，急性型神経Behçet病で基底核に複数のring enhancementを伴うT1低信号，T2高信号域を認めた症例に対し，造影効果のある部位とない部位から生検を行っており，両方の部位に血管周囲腔への炎症細胞浸潤を認めたと報告しているが，造影の有無の違いについての背景病理は示されていない[15)]．

なお，本例は臨床的長期間寛解を保っていたにもかかわらず，病理学的にTリンパ球の血管周囲へのカッフィングを少数ながら認め，染色質融解を伴う神経細胞を病変部に認めた点は，Behçet病の寛解期においても病勢がくすぶっていることを示唆する所見と考えられた．

まとめ

1990年の65歳時に口腔内アフタでBehçet病を発症し，70歳で急性型神経Behçet病を合併，MRIで橋にring enhancementを伴う病変を認めたがステロイド治療が奏効し，その後10年間の寛解を得た死亡時80歳男性剖検例を報告した．急性型神経Behçetの病理報告は生検例と急性期に死亡した剖検例が散見される[15)16)]のみで，本例のように長期追跡例の報告はなく，初めての例と考えられた．また，ring enhancementの部位の病理所見は，炎症と内部の出血とする当時の画像所見の解釈を支持する結果であった．

本論文の要旨は2005年11月26日の第175回日本神経学会関東地方会(東京)，2006年1月7日の第82回関東臨床神経病理懇話会(東京)で発表した．

本例の経過をまとめるにあたって，ご助言をいただいた東京大学神経内科・百瀬義雄先生，北里大学膠原病・リウマチ・感染内科・広畑俊成教授に深謝する．

文　献

1) Kidd D, Steuer A, Denman AM, et al. Neurological complications in Behçet's syndrome. Brain 1999；122：2183-94.
2) 広畑俊成．神経ベーチェット病の病態．臨床神経2001；41：1147-9.
3) 百瀬義雄，喜多也寸志，坂東充秋，ほか．MRI上，脳幹部にring enhancementを呈した神経Behçet病．神経内科 1998；49：190-2.

4) 広畑俊成. 神経ベーチェット症候群. Brain Medical 1991；3：375-81.
5) Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. Lancet 1990；335：1078-80.
6) 稲葉午朗，岸 いずみ. 神経ベーチェット病. 最新医学 1988；43：321-8.
7) Phillips DL, Scott JS. Recurrent genital and oral ulceration with associated eye lesions；Behçet's syndrome. Lancet 1955；268：366-71.
8) Pallis CA, Fudge BJ. The neurological complications of Behçet's syndrome. A M A 1956；75：1-14.
9) Wadia N, Williams E. Behçet's syndrome with neurological complications. Brain 1957；80：59-71.
10) 田中 健，浅野昭一. 中枢神経症状を伴うBehçet氏症候群，いわゆるneuro-Behçet's syndromeの2例及びその文献的考察. 神経進歩 1960；4：184.
11) 池田久男，石野博志，岡本 繁，ほか. Behçet病の中枢神経症候—とくにその精神症状の特徴について—. 精神医学 1975；17：1295-305.
12) 稲葉午朗，青山順子，清水 保. Neuro-Behçet症候群. 神経進歩 1980；24：119-29.
13) 広畑俊成. 膠原病における中枢神経病変. 日本臨牀 1999；57：159-62.
14) 調 輝男. 神経ベーチェット症候群. 臨床神経病理. 改訂3版. 京都：金芳堂；1992. p. 96
15) Imoto H, Nishizaki T, Nogami K, et al. Neuro-Behçet's disease manifesting as a neoplasm-like lesion—case report. Neurol Med Chi(Tokyo) 2002；42：406-9.
16) Matsuo K, Yamada K, Nakajima K, et al. Neuro-Behçet disease mimicking brain tumor. AJNR Am J Neuroradiol 2005；26：650-3.

＜Abstract＞

A case of neuro Behçet disease presenting with ring enhancement in the pontine base by MR imaging. A long-term follow-up study with autopsy.

by

Yoshio SAKIYAMA, M.D., *Takahiro SAITO, M.D., *Masatoshi YOSHINO, M.D., **Yuko SAITO, M.D., Ph.D. & Shigeo MURAYAMA, M.D., Ph.D.

from

Department of Neuropathology, Tokyo Metropolitan Institute of Gerontology, Itabashi, Tokyo 173-0015, Japan, and Departments of *Neurology and **Anatomical Pathology, Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan.

We report an autopsy case of acute neuro-Behçet disease in remission after long-term follow up. At the age of 65 years, the patient first presented with multiple aphthous ulcers in the mouth and painful erythema nodosum on the legs. At the age of 70, he was admitted because of headache, character change and gait disturbance. He had a bruise-like rash on the face, folliculitis on the arms, and old ulcers on the scrotum, in addition to painful erythema nodosum on the legs. Neurological examination disclosed euphoric and childish character with right hemiparesis. MRI showed ring enhancement in the left pontine base. Pleocytosis and elevated IL6 level were detected in the cerebrospinal fluid. He showed a good response to high-dose prednisolone therapy. He had not experienced overt relapse until he died of aspiration pneumonia at the age of 80. The autopsy was performed with a postmortem interval of 8 hours. The brain weighed 1086g before fixation. Macroscopically, multiple brownish atrophic lesions involved the left base of the pons, midbrain, hypothalamus, internal segment of globus pallidus and occipital cortex. Histologically, the lesions presented with rarefaction of the tissue with peripheral demyelination, and central deposition of hemosiderin, but there was little lymphocytic cuffing around the small vessels. Rare and sparse lymphocytic cuffing around small vessels suggested that this case maintained remission after single acute attack and was clearly different from chronic progressive subtype of neuro Behçet disease. Our study confirmed previous speculation (Momose et.al. 1994) that the ring enhancement in MR imaging at the onset reflected focal inflammatory changes accompanying internal venous-type hemorrhage. To our knowledge, this is the first autopsy case representing the remission stage of acute neuro-Behçet disease.

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後頭葉の糖代謝が低下し，臨床症状より DLBD が疑われた 76 歳男性例

金澤俊郎　織茂智之　服部　亮
足立朋子　笠井陽介　岡　輝明
石井賢二　村山繁雄　河村　満

BRAIN and NERVE
第 60 巻　第 10 号　別刷
2008 年 10 月 1 日　発行

医学書院