A Genetic Demyelinating Disease Globoid Cell Leukodystrophy:
Studies with Animal Models

KINUKO SUZUKI, M.D.

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Krabbe's disease or globoid cell leukodystrophy (GLD) is a familial neurologic disorder affecting infants, neuropathologically characterized by the presence of numerous abnormal macrophages (globoid cells), severe gliosis and paucity of myelin in the central nervous system. Unique neuropathological features with presence of abnormal cells were first described by Danish neurologist Dr. Knud Krabbe in 1916, in two infants who died of an acute infantile familial diffuse sclerosis of the brain (1). Later, Collier and Greenfield (2) introduced the term "globoid" to describe these abnormal cells in the white matter. Since then, numerous investigations focusing on the pathogenesis of this disease have been conducted. Deficiency of an activity of the enzyme galactocerebrosidase (galactocerebroside β galactosidase) was discovered as a cause of GLD in 1970 (3) and more than 20 years later, finally this year (in 1993), the cDNA for galactocerebrosidase was cloned (4). The next obvious steps will be to identify the mutations causing GLD and also to investigate the role(s) of this enzyme in normal myelination and myelin maintenance. [A detailed review of Krabbe disease has been described elsewhere (5).]

In this brief review, I would like to describe the historical aspect of the investigation of Krabbe disease, in particular focusing on the studies using animal models, emphasizing an important role of animal models to study the pathogenesis of genetic diseases.

The first step to elucidate the pathogenesis of this disease was taken by Dr. James Austin who found a high concentration of galactocerebroside in the isolated globoid cell fraction (6). With injection of purified galactocerebroside in the adult rat brain, globoid cell reactions identical to those found in the brain of the patients with GLD could be produced (7–10). Ultrastructural features of the storage materials in the globoid cells in the GLD patient (11) and those experimentally produced with injection of the galactocerebroside were identical (9, 10).

Thus formation of globoid cells, a unique feature of GLD, can be well explained as macrophage reaction to galactocerebrosidase, which could not be hydrolyzed due to the genetic defect in an activity of galactocerebrosidase β galactosidase (3).

D'Agostino and co-workers (12), on the basis of detailed neuropathological examination of three autopsy cases, suggested that GLD is a progressive demyelinating disorder. He and his co-workers formulated the following sequence of events in pathological lesions of GLD: 1) globoid cells appeared in an early stage of demyelination, 2) they increased with progression of degeneration of the white matter, and 3) they disappeared in the late terminal stage when degeneration and gliosis became pronounced (12). Galactocerebrosidase induced globoid cell reaction but did not cause any demyelinating lesions. Thus, demyelination has to be explained by other mechanisms. Subsequently, the tissue of patients with GLD was also found to have deficient enzyme activity to cleave galactose from three other lipids, namely galactosylsphingosine (psychosine) (13), monogalactosyl diglyceride (14) and lactosylceramide (15). Available data indicated that these enzymes were the same single enzyme, galactosylceramidase, acting on the terminal galactose of these four natural substrates. Thus, brain tissue reactions to these four natural substrates were tested. The results indicated that galactocerebroside and lactosylceramide induced granulomatous reaction with multinucleated cells at the site of injection, while galactosylsphingosine (psychosine) was found to be highly toxic to the tissue and induced diffuse degeneration of white matter (16). Thus, the following hypothesis was proposed: "In GLD, the toxic metabolite of galactosylceramidase, psychosine, accumulates in the myelin forming cells, namely oligodendrocytes and Schwann cells, causing cellular dysfunction and degeneration, and eventual myelin degeneration" (13). Psychosine was found to be negligible in the normal brain but was increased at least ten times in the brain of the patient with GLD (17). If indeed psychosine has a significant role in the white matter degeneration in GLD, its concentration in the brain should increase in parallel with the progression of white matter degeneration. For obvious reasons, chronological psychosine determination in human brain is very difficult, if not impossible. An animal model is very useful for such chronological study.

GLD is a rare disease in humans but is found in several
mammalian species. Canine GLD was the first model described by Fankhauser et al in 1963 (18). The disease occurs commonly among two breeds of dogs (Cairn terriers and West Highland terriers) (19–22), although sporadic cases have been reported in other breeds. Roszel and co-workers (23) reported PAS-positive globoid cells in the cerebrospinal fluid in canine GLD, an important observation supportive of globoid cells being of peripheral macrophage origin. Furthermore, electron microscopic study revealed galactocerebroside inclusions in the perikarya of oligodendrocytes, in addition to globoid cells, a feature consistent with metabolic perturbation of oligodendrocytes in GLD (24).

Deficiency in activity of galactosylceramidase was demonstrated in most of these canine models and thus they are morphologically as well as biochemically authentic models of human disease. These large animal models are very useful for the clínico-pathological investigations but maintenance is very expensive. Unfortunately, the colony of canine GLD which was maintained for several years at the University of Minnesota by Dr. Thomas Fletcher was discontinued.

Murine model twitcher was less expensive to maintain and has great advantages for the laboratory use because of its size, availability of littermate controls, rapid reproduction, etc. This murine mutant was discovered in the CEJ strain of mouse in the Animal Researches Division of the Jackson Laboratory, Bar Harbor, Maine, in 1976. Current breeder stock is on the C57BL strain background (C57BL/6J-twi), which was created by intercrossing heterozygous twitcher mice with CE/J and with C57BL strain background. The homozygous twitcher (twi/twi) was normal clinically until about 20 days of age when gradual wasting of the trunk and limbs became apparent. Body weight gain gradually retarded, in association with generalized tremulousness, particularly marked in head, progressive weakness of limbs, hindlimbs being more affected than forelimbs, and the mice eventually became totally paralyzed and died. In the original report, the F2 generation of heterozygous CE/J male and C57BL female mice survived up to 3 months of age (25), but the lifespan of inbred twi/twi (C57BL/6J-twi) obtained from the Jackson Laboratory rarely survive beyond 45 days of age. In this murine model, activity of the enzyme galactosylceramidase was deficient (26) and neuropathological features were almost identical to those of human GLD. Unlike human cases, however, peripheral nerves tended to be more severely involved than the brain in the twi/twi (25–28).

There has been a question whether early myelination is normal in leukodystrophy. Developmental studies of the spinal cord showed no differences, among homozygous, heterozygous and normal control littermate mice, in the pattern and progression of early myelination of the spinal tracts (29).

With all these available data, the following hypothesis was formulated for the pathogenesis of GLD: 1) early myelination progresses normally up to a certain stage, 2) disruption of normal myelin turnover due to deficient activity of lysosomal hydrolase galactosylceramidase, 3) degeneration of myelin-forming cells, oligodendrocytes and Schwann cells, due to an accumulation of a toxic metabolite of galactosylceramidase, galactosylsphingosine (psychosine), 4) demyelination secondary to degeneration or dysfunction of myelin-forming cells, and 5) macrophages infiltrate in the nervous tissue in response to demyelination and unmetabolized galactocerebroside.

Three genotypes of mice (twi/twi; +/-twi; +/-) were identified by an assay of galactosylceramidase activity before twi/twi mice developed clinical symptoms (26) and the pattern of myelination was studied in the three nerve fiber tracts, gracile, cuneate and corticospinal, in the upper cervical spinal segment. There were no differences in the numbers of glial cells and myelinated fibers and thickness of myelin sheaths vs axonal diameter up to day 20 when hypomyelination, in particular in the fibers with large diameter, became apparent in twi/twi (29).

Similarly in the sciatic nerve, early normal myelination up to day 15, followed by hypomyelination, was described (30, 31). Consistent with these morphological data, activity of the enzyme UDP-galactose:ceramide galactosyltransferase, which catalyzes the last step of galactosylceramidase synthesis in oligodendrocytes, was normal up to day 15, fell slightly at day 20 and reduced drastically with progression of demyelination (32). Also, a composition of galactolipids in the brain, spinal cord and sciatic nerve of twi/twi was normal during early myelination (33).

With progression of disease, psychosine concentration increased in the twi/twi brain (34). Severity of neuropathological lesions in the twi/twi CNS and PNS paralleled the degree of psychosine accumulation (31, 35, 36). Ultrastructural study of the twi/twi spinal cord revealed typical inclusions of GLD in the perikarya and the processes of oligodendrocytes as early as day 10 prior to any detectable neurological signs and symptoms. After day 25–30, degenerating oligodendrocytes and myelin sheaths were noted (37). Also with a modified iron histochemical stain, morphologically aberrant oligodendrocytes were demonstrated in the twi/twi brain (38).

To test the hypothesis that degeneration of oligodendrocytes is the consequence of their own metabolic perturbation, oligodendrocytes were isolated from twi/twi and control +/- mice and cultured in vitro. By 4 days in vitro (DIV), the morphology of cultured oligodendrocytes was similar regardless of genotype, although distal branches of oligodendrocytic processes in twi/twi were somewhat sparse in comparison with those of controls. Retardation of the development of distal branches and membranous sheet-like expansion of twi/twi oligodendro-
cytes became obvious by 10 DIV and eventually many tvi/tvi oligodendrocytes were degenerated. Characteristic GLD inclusions were detected in these degenerating oligodendrocytes (39, 40).

In the tvi/tvi peripheral nerves, Schwann cells were numerous and, unlike the CNS where degeneration of oligodendrocytes was observed with progression of the disease process, degeneration of Schwann cells was not observed (30). However, isolated Schwann cells showed a decreased rate of proliferation compared with heterozygous (+/tvi) and normal (+/+) mice and progressively declined with age (41, 42). Furthermore, Schwann cells isolated from the adult tvi/tvi nerve progressively showed degenerative changes with accumulation of the GLD inclusions, while those isolated from young tvi/tvi before demyelination showed lesser degrees of morphological changes (43). These studies indicate that, indeed, metabolic perturbation of tvi/tvi Schwann cells was present but was masked by the presence of microenvironmental factors such as Schwann cell mitogenic factors released by infiltrating macrophages (44).

When the disease process progressed, globoid cells, PAS-positive phagocytic macrophages, increased in areas of demyelination. There had been a long debate as to the nature of globoid cells, namely intrinsic microglial origin or extrinsic infiltrating monocyte macrophages. These globoid cells were morphologically identical to macrophages infiltrating in the PNS, and with intraventricular injection of colloidal carbon, some of these cells in the CNS were found to contain carbon particles. Therefore, now it is generally accepted that these globoid cells are infiltrating hemogenous macrophages. On frozen sections of unfixed CNS tissue, Mac-1 immunoreactivity was demonstrated only on these macrophages (45). When the CNS tissue was fixed with periodic lysine parafomaldehyde (PLP), Mac-1 immunoreactivity was also detected in the plasma membrane of intrinsic microglial cells. Strong immunoreactivity was demonstrated in this latter preparation in reactive microglial cells as well (46). Thus, it appeared that in addition to infiltrating macrophages in response to demyelination, reactive (or activated) microglia also participated in the neuropathology of GLD. Inclusions typical of GLD were also found in these reactive microglia in the gray matter.

Unlike experimental autoimmune encephalomyelitis (EAE), GLD is not immune-mediated, but a genetic demyelinating disease. However, in both CNS and PNS, the major histocompatibility antigen (MHC) Class II-expressing cells appeared with accompanying CD4 T cell infiltration to a certain extent. Expression of MHC Class II molecules was transient, however. MHC Class II-expressing cells appeared to be expressed during an early stage of demyelination and disappeared despite continuous demyelination. This feature may suggest participation of some immune mechanism in the development of the demyelinating lesion, even in a primary genetic demyelinating disorder (47).

In addition to the study of the pathogenesis of the diseases process, authentic animal models are very useful for possible therapeutic manipulation. In sphingolipidoses such as GLD, deficient enzymes are known. Therefore, the obvious therapy of choice is an enzyme replacement therapy. Enzyme replacement therapy by allogeneic bone marrow transplantation has been performed in many human sphingolipidoses, although its therapeutic effectiveness on sphingolipidoses affecting the CNS is still controversial. Bone marrow transplantation (BMT) using authentic animal models will provide important information to evaluate its effectiveness as a treatment for these types of genetic diseases affecting the nervous system. Two groups of investigators independently conducted BMT using the twitcher model (48, 49). The basic experimental protocols in both groups were almost identical. Essentially, twitcher homozygotes were identified before clinical onset of disease by enzyme assays, and following total body irradiation allogeneic BMT was performed. More recently, the alkylating agent with profound myelosuppressive effect, Busulfan (Burroughs-Wellcome Company, Research Triangle Park, NC), was used successfully in replacement of irradiation (50). The twitcher which received BMT survived longer and neurological symptoms improved, but growth retardation still remained. The twitcher which received BMT survived over 100 days, while untreated twitcher rarely survived beyond 45 days of age. Globoid cells containing typical galactosylceramide inclusions were replaced by foamy donor macrophages, myelin degeneration ceased and remyelination took place in the CNS and PNS of these mice (51, 52). The psychosine level was drastically decreased. Activity of galactosylceramidase increased gradually in the brain although it never reached the normal level. In contrast to slow recovery in the CNS, enzyme levels in the spleen and bone marrow reached as high as the control level soon after the BMT. This slow recovery of the CNS is most likely due to the presence of the blood-brain barrier, which remained intact despite demyelination and infiltration of macrophages (53). Ultrastructural study revealed, however, the presence of GLD inclusions in oligodendrocytes and Schwann cells in the remyelinating nervous system, and a gradual increase of psychosine has been noted in the peripheral nerves (51). These results suggest that BMT ameliorates neuropathology and clinical symptoms to some extent but it may not cure the disease process completely. In the future, gene therapy may be an obvious treatment of choice for genetic diseases affecting the nervous system. For exploratory studies of ultimate gene therapy of these genetic diseases, the importance of the authentic animal models cannot be overemphasized.
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