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GENETIC DEFECTS OF LYSOSOMAL FUNCTION IN ANIMALS

Shutish C. Patel

Neurology Service, Veterans Administration Medical Center, Newington, Connecticut 06111; and Department of Neurology, University of Connecticut School of Medicine, Farmington, Connecticut 06032

Peter G. Pentchev

Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Building 10, Room 3D-12, Bethesda, Maryland 20295

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INTRODUCTION

Defects in the lysosomal apparatus may arise as a consequence of both inherited and acquired abnormalities. Genetic mutations account for a majority of disease-producing lysosomal lesions in mammals. Metabolic disturbances of this nature are most often due to mutations in genes encoding degradative lysosomal enzymes. Substrates of deficient enzymes accumulate in enlarged lysosomes and lead to an array of secondary disruptions in cellular functions. Each specific enzyme deficiency results in a distinct lysosomal storage disorder.

Some lysosomal diseases are not related to a defect in a degradative lysosomal enzyme. For example, in cystinosis (33, 97) and in Salla disease (33) the lysosomal storage disorder occurs as a result of a defect in the transport of cystine and sialic acid, respectively, out of lysosomes. Many additional disturbances of lysosomal function have been produced by nongenetic alterations of lysosomal properties, and animal models for such conditions have been established (18, 49, 61, 95, 116). Most of the clinically relevant defects in lysosomal function are represented by lysosomal storage diseases of genetic origin, however, and their animal counterparts are the subject of this review.

During the past decade, many pathologic states in animals have been studied. There are compelling reasons to catalog and investigate such diseases. From a veterinary point of view, the culling of defective genes strengthens breeding programs of all domestic animals including cats, dogs, horses, cattle, and goats. For example, mannosidosis in Angus cattle was the most common lysosomal storage disease in any species, affecting 2500 cattle per year in New Zealand, before measures were instituted to control breeding of carriers (54). In this instance, a genetic disorder had national impact on food production and monetary revenues.

It is from an entirely different perspective, however, that the study of these genetic disorders in animals has had its greatest impact on humans. For clinicians and researchers engaged in the diagnosis, management, and therapy of human genetic disorders, many complex and limiting factors exist. Affected individuals often have shortened life spans. Reliable diagnostic procedures can be complex and are frequently difficult to establish in the laboratory. Creation of each new therapy is fraught with repeated trials and uncertain outcome. For those conditions amenable to therapy, there is often only a short window of time in which to make therapeutic decisions. Alternative therapies may have significant morbidity and mortality. Moreover, establishing the success or failure of a therapeutic strategy requires a long period of observation and investigation. Bone marrow transplantation, enzyme replacement, and now gene replacement are still in the research phases of their development. Therefore, the availability of animal counterparts for

human disorders provides powerful and vital tools not only for investigating molecular pathogenesis but also for designing and testing potential therapeutic strategies in humans.

Several comprehensive accounts exist of the many animal analogs of human inborn errors of metabolism (3, 4, 11, 15, 21, 24, 80). The use of such animal models for the study of analogous human disorders has sometimes been hampered for a variety of reasons. First, the model itself may not be truly representative of the human disease. This pitfall can be circumvented by strictly restricting definitions of the animal model so that they are congruent with the biochemical deficiency characterizing the human disorder. Second, experimental constraints may be imposed by the species concerned. Some species, particularly higher primates, have been difficult to line breed, and the cost of maintaining many such animals can be prohibitive. In some instances, poor breeding characteristics of the relevant species have been a major impediment. Not surprisingly, then, many discoveries of animals with diseases analogous to human disorders have not led to further contribution. Conversely, mutations in mice are often comparatively easy to exploit for research purposes. For example, over the past five years progress in the understanding of two human lysosomal storage disorders, Krabbe's disease (108) and Niemann-Pick disease, type C (10), stems largely from studies of their murine counterparts.

This article reviews the major animal models of human lysosomal storage disorders and emphasizes those diseases that have provided important information on the pathophysiology of their human counterparts.

ANIMAL MODELS AND THEIR CLASSIFICATION

In a topic as broad as genetic defects of lysosomal function, subdivision is often arbitrary because many disorders are not discrete entities of specific excess biochemical storage. Often, there is accumulation of multiple storage products that overlap several categories. Given these limitations, we discuss five general categories of lysosomal disorders in animals, grouped by major storage products: sphingolipidoses, glycogenoses, glycoproteinoses, mucopolysaccharidoses, and proteinoses. Table 1 catalogs the major animal models of human lysosomal disorders.

Sphingolipidoses

The sphingolipidoses are a group of diseases caused by defective lysosomal degradation of complex membrane molecules known as sphingolipids. All sphingolipids have a common backbone consisting of sphingosine, an amino diol, and a long unsaturated hydrocarbon chain that is acylated via an amide linkage at the second carbon. This compound, common to all sphingolipids, is known as ceramide. The hydroxyl group at the first carbon position can be

Table 1 Catalog of animal models of human lysosomal storage diseases

Disease	Biochemical defect	Mode of inheritance	Species	Reference number
Sphingolipidoses				
G_{M1}-Gangliosidosis	G_{M1} β-galactosidase	AR[a]	Cat	4, 7, 35
			Dog	92
			Cow	25
G_{M2}-Gangliosidosis				
Type 1	*N*-acetyl hexosaminidase A	AR	Dog	62
Type 2	*N*-acetyl hexosaminidase A & B	AR	Cat	20
Type 3	Partial deficiency of hexosaminidase A & B	AR	Swine	87
Gaucher's disease, type 2	β-Glucocerebrosidase	AR	Dog	114
Krabbe's disease	Galactosylceramidase	AR	Mouse	27
			Cat	53
			Dog	8, 31, 52, 12
			Sheep	88
Niemann-Pick disease, type A	Sphingomyelinase	AR	Cat	5, 125
			Dog	12
Niemann-Pick disease, type C	Lysosomal storage of cholesterol and sphingomyelin; specific defect presently unknown	AR	Mouse	72
Glycogenosis				
Glycogenosis, type II (Pompe's disease)	Acid α-glucosidase	AR	Cow	93
			Sheep	69
			Cat	96
			Dog	73, 119
Glycoproteinoses				
α-Mannosidosis	α-Mannosidase	AR	Cow	54
β-Mannosidosis	β-Mannosidase	AR	Cat	106
			Goat	36, 56, 57
Mucopolysaccharidoses				
MPS I H; Hurler syndrome	α-L-Iduronidase	AR	Cat	38
MPS I H/S	α-L-Iduronidase	AR	Dog	103
MPS VI (Maroteaux-Lamy)	Arylsulfatase B	AR	Cat	40
MPS VII	β-Glucuronidase	AR	Dog	37
Overlapping Storage Disorder				
Ceroid lipofuscinosis	?lysosomal proteases	AR	Sheep	54
			Dog	22, 65, 112

[a]AR = autosomal recessive.

substituted with neutral sugars (usually hexoses) to yield cerebrosides. When one or more sialic acids are attached to any one of the sugars, a charged compound (a ganglioside) is produced. Groups other than sugars may be attached to ceramide. Thus, for example, sphingomyelin is formed when phosphorylcholine is attached to ceramide. The addition of sulfate to galactosylceramide yields sulfatide. These compounds, sphingomyelin, galactosylceramide, and sulfatide, are especially enriched in myelinated nervous tissue.

Eleven human sphingolipidoses have been described, each caused by a catalytic deficiency of a specific lysosomal hydrolase. Animal analogs for several human sphingolipidoses have been described (Table 1) and are discussed below.

GAUCHER'S DISEASE, TYPE 11 Gaucher's disease has been described in dogs (30, 114). The defect was shown to be a deficiency in lysosomal β-glucocerebrosidase and accumulation of glucosylceramide and glucosylpsychosine. Further work on this model has been precluded by difficulties in establishing a breeding colony.

NIEMANN-PICK DISEASE, TYPE A Animal models of Niemann-Pick disease, type A, have been described in cats (5, 125) and in a strain of poodles (12). The canine disorder has not been characterized beyond an initial descriptive study. Feline sphingomyelin lipidosis has been described in a strain of Siamese cats by Wenger et al (125). Affected cats become symptomatic at 4–5 months of age and display tremors, ataxia, hind-limb weakness, anorexia, and apathy. There is a total deficiency of acid sphingomyelinase in leukocytes, liver, and brain. Activity of the neutral (pH optimum 7.4), magnesium-stimulated microsomal sphingomyelinase is normal in brain. Lipid analysis of affected animals revealed a tenfold increase in sphingomyelin and cholesterol in the liver and an excess of the gangliosides G_{M2} and G_{M3} in brain. More recently, Baker et al described a similar disorder in Balinese cats (5). Clinical, morphologic, and biochemical findings are similar to those described in the Siamese cats.

G_{M1}-GANGLIOSIDOSES G_{M1}-gangliosidosis is caused by a deficiency of G_{M1} ganglioside β-galactosidase and leads to excessive accumulation of the ganglioside G_{M1} (76). Because the enzyme shows a multiple substrate specificity, additional metabolites that have a terminal β-D-galactose can fail to be catabolized and can accumulate in tissues of affected subjects. Thus, keratan sulfate, oligosaccharides, glycoproteins, and asialo-G_{M1} can accumulate in this disorder (75).

Animal models of G_{M1} gangliosidosis have been reported in cats (4, 7, 35), in a strain of beagles (92), and in Friesian cattle in Ireland (25). Most of the studies thus far have focused on mutant Siamese cats (4) and mixed-breed beagles (92). The primary defect in both these animal models is a deficiency

in acid β-galactosidase activity. Holmes & O'Brien (45) found a pattern of hepatic oligosaccharide accumulation in the feline disorder similar to that observed in human G_{M1} gangliosidosis, with the notable difference that the structure of the accumulating oligosaccharides in feline liver showed an extra *N*-acetylglucosamine residue at the reducing terminus. Residual acid β-galactosidase activity in the liver of affected cats was only 10% of normal. The partially purified residual hepatic enzyme activity had altered physical and kinetic properties compared with wild-type feline liver β-galactosidase and did not show immunologic cross-reactivity to antibodies against the wild-type enzyme (44). In contrast, the residual β-galactosidase activity in canine G_{M1} gangliosidosis liver is more severely reduced (1% of normal) but has physical and kinetic properties comparable with wild-type dog liver β-galactosidase with which it shows immunologic cross-reactivity (94). These findings suggest that these two models of G_{M1} gangliosidosis are caused by different genetic lesions. The feline disorder most likely represents a structural mutation in the β-galactosidase gene, whereas the canine disorder could be caused by a regulatory gene mutation.

G_{M2} GANGLIOSIDOSIS The G_{M2} gangliosidoses (76) are a heterogeneous group of disorders that show excessive storage of the ganglioside G_{M2} because of a deficiency of β-hexosaminidase activity. Although newer G_{M2} gangliosidosis variants continue to be discovered, especially in adult patients with neurodegenerative conditions, several major categories of this disease have been well characterized and animal models for some are known. Feline G_{M2} gangliosidosis has been the most intensively studied G_{M2} gangliosidosis in animals. The disorder was first reported by Cork et al in 1977 (20). Beta-D-*N*-acetylhexosaminidase activity is reduced to about 1.0% of normal in brain, liver, and cultured skin fibroblasts of affected animals. Both major electrophoretic forms of the enzyme are deficient, thus making the feline disorder analogous to Sandhoff's form of human G_{M2} gangliosidosis (76). Heterozygous cats display intermediary levels of hexosaminidase deficiency, thereby indicating an autosomal recessive pattern of inheritance.

KRABBE'S DISEASE Krabbe's disease in humans is a rapidly progressive, sphingolipid storage disorder of infants, caused by deficient activity of the lysosomal hydrolase galactocerebroside β-galactosidase (108). Galactosylceramide accumulation in the brain is associated with the disappearance of oligodendroglia and loss of myelin. Characteristically, numerous multinucleated globoid cells accumulate in the white matter of affected individuals, hence the name "globoid cell leukodystrophy" (GLD). Animal models for GLD have been described in domestic short-hair cats (53), polled Dorset sheep (88), beagles (52), poodles (128), Bluetick coonhounds (8), West Highland and Cairn terriers (31, 32, 47, 109), and a mutant strain of mice

known as "twitcher" (27). Because considerable progress has been made in the understanding of human GLD from studies conducted on the twitcher mouse, this murine mutant is discussed below in greater detail.

This particular mouse (gene designation *twi*) was discovered as a spontaneously occurring mutant in a colony of mice at the Jackson Laboratory in 1976. The disorder is transmitted as an autosomal recessive trait. Twitcher mice become symptomatic at approximately 30 days of age; they manifest tremulousness, progressive muscular weakness, and difficulty with head holding. These symptoms are inexorably progressive and lead to loss of body weight and muscle wasting. The hind limbs are more severely affected, and in advanced stages the animals become paralyzed. During the later stages, a dorsal kyphosis produces a hump in the lower cervical to upper thoracic region. Death invariably ensues by 12 weeks of age. Morphologic examination of the brain shows patchy areas of myelin loss with mild astrocytic gliosis. Scattered within the white matter are abnormal, PAS-positive cells, which are often multinucleated (27). Ultrastructural examination reveals polygonal paracrystalline inclusions and twisted tubules within these cells. Similar findings are present in the peripheral nervous system. The activity of galactocerebroside β-galactosidase is severely deficient in the twitcher mouse, thus establishing this murine mutant as an analog of human GLD (64). The genetic status of presymptomatic twitcher mice can be conveniently established by assay of galactocerebroside β-galactosidase activity in the clipped tails of the animals (63). In spite of the close biochemical and morphologic resemblance between the twitcher mouse and human GLD, differences exist (50).

Glycogenoses

Glycogen storage diseases (GSD) are inborn errors of glycogen metabolism. Hers (41) initially showed that GSD II (Pompe's disease; generalized glycogenosis) is caused by a deficiency of lysosomal α-1,4-glucosidase (EC 3.2.1.20). Three forms of the disorder have been recognized: infantile, late infantile-juvenile, and adult. Infantile GSD II is a fatal autosomal recessive disorder in which glycogen accumulates in heart, skeletal muscle, brain, liver, and kidney. Affected patients display severe hypotonia and massive cardiomegaly. Most patients die in the first year. Patients with the late-infantile, or juvenile, form of the disorder have a slowly progressive myopathic conditions without cardiac involvement. GSD II in adults occurs as a chronic myopathy with little or no cardiac involvement (29).

Animal models of glycogen storage diseases and their use as models of analogous human conditions have been reviewed (118). Generalized glycogenosis has been described in the dog (73, 119–122), cat (96), sheep (69), and an Australian line of shorthorn cattle (28, 93). Biochemical studies have been limited to bovine generalized glycogenosis (93) and to the disorder found in the Lapland dog (122). The bovine disorder has characteristics of

both the infantile and juvenile forms of the human disease. In brain and spinal cord of the calves, acid α-glucosidase activity is depressed, and there is marked accumulation of glycogen in all tissues. The glycogen is both membrane bound and free within the cytoplasm. Walvoort has characterized the clinical and biochemical phenotype of glycogen storage disease type II found in a family of Lapland dogs (119). The canine disorder is similar to infantile human GSD II. There is generalized glycogen storage, particularly in skeletal, esophageal, cardiac, and smooth muscle. Esophageal dilatation that leads to megaesophagus and frequent vomiting is common in canine GSD II. Affected animals have elevated glycogen content in heart and skeletal muscle but not in the liver. Acid α-glucosidase activity is markedly reduced in heart, skeletal muscle, liver, and cultured tongue fibroblasts (121). Immunoreactive α-glucosidase protein that is catalytically inactive can be demonstrated in tissues from affected dogs; this finding indicates that the disorder is caused by a structural mutation in the α-glucosidase gene (122).

Glycoproteinoses

The glycoproteinoses include a group of disorders that clinically resemble mild mucopolysaccharidosis. Four human disorders caused by defective degradation of glycoproteins have been characterized: mannosidosis, fucosidosis, sialidosis, and aspartylglycosaminuria (6). Animal glycoproteinoses include α-mannosidosis in Angus cattle (55) and in a strain of domestic cats (13, 106) and β-mannosidosis in Nubian goats (56, 57). In bovine mannosidosis, affected calves display a progressive neurodegenerative disorder characterized by ataxia; they usually die within a year. Lysosomal α-mannosidase is variably deficient in the plasma and tissues of affected animals. Plasma and leukocyte activity is severely deficient, whereas 8–15% of normal activity is present in pancreas, liver, brain, and other organs of affected cattle (14). The residual enzyme shows immunological cross-reactivity with wild-type bovine α-mannosidase, is more thermolabile, and has reduced substrate-binding capacity. Heterozygotes have 35–38% of normal α-mannosidase activity, which allows appropriate carrier detection.

Feline α-mannosidosis has been reported in a strain of domestic cats (106). The disorder resembles the infantile form of human α-mannosidosis (6). Acidic α-D-mannosidase activity is severely deficient in the brain, kidney, and liver of affected animals (2–5% of normal); excretion of mannose-rich oligosaccharides in the urine is increased 19-fold over comparable controls (13). The residual α-mannosidase activity in the lysosomal fraction from liver of affected cats is thermolabile and has altered kinetic properties (89). Whether this residual activity represents altered properties of the mutant enzyme or minor α-mannosidase activity not related to the major lysosomal enzyme has to be established.

An interesting reversible syndrome of α-mannosidosis can be induced by feeding normal cats the plant-derived indolizidine alkaloid swainsonine, which specifically inhibits the lysosomal hydrolase α-mannosidase (117). Affected animals develop morphologic and biochemical changes similar to the genetically inherited condition.

β-Mannosidosis caused by deficient lysosomal acid β-mannosidase activity was extensively characterized in goats (16, 36, 56–60, 70) prior to the discovery of a similar deficiency state in humans (19, 126). The animal disorder was first described in inbred Nubian goats as a neurovisceral storage disorder by Jones et al (56) in Michigan and independently by Hartley & Blakemore (36) in Anglo-Nubian goats in New South Wales, Australia. Human β-mannosidosis was not documented until 1986 when severe deficiency of β-mannosidase in leukocytes, plasma, and cultured fibroblasts was described separately in two patients (19, 126). Interestingly, one patient had a concomitant complete deficiency of sulfamidase (126). The pattern of inheritance in both goats and humans is autosomal recessive. The caprine disorder is clinically more severe than the recently described human cases. Affected goats are symptomatic at birth and display moderate to marked intention tremor, abnormal eye movements resembling pendular nystagmus, deafness, hyperextension of the pastern joints, carpal contractures, thickened skin and, to a varying degree, a dome-shaped skull (57). The brains of affected goats display hypomyelination with decreased numbers of oligodendrocytes. Axonal spheroids are prominent and the remaining oligodendrocytes show cytoplasmic vacuolation (57). The two patients with β-mannosidosis have mild mental retardation. Severe hearing loss, an invariant feature of goat mannosidosis, was not described. The patient with combined β-mannosidase and sulfamidase deficiency had mild facial dysmorphism (126). In goat β-mannosidosis, β-mannosidase activity is absent in brain and cultured fibroblasts, whereas the hepatic activity is only partially deficient (40% of normal) (16, 59). This residual activity might represent nonlysosomal (neutral) β-mannosidase (16). The tissue storage product in caprine mannosidosis is a trisaccharide with the structure Man-β-(1→4)GlcNAc-β-(1→4)GlcNAc (70). In contrast, human β-mannosidosis is associated with the urinary excretion of the disaccharide Man-β-(1→4)GlcNAc (19, 126). This difference in the storage compounds is thought to be responsible for the phenotypic differences between caprine and human β-mannosidosis (19, 126).

Mucopolysaccharidoses

The mucopolysaccharidoses (MPS) are a group of inherited lysosomal storage diseases caused by inborn errors of glycosaminoglycan (GAG) metabolism (26, 71). In humans, the MPS are characterized by dysostosis multiplex,

hepatosplenomegaly, mental retardation, facial dysmorphism, lysosomal accumulation of GAG, urinary excretion of GAG, and metachromatic granules in peripheral leukocytes.

Examples of MPS in animals have occurred mainly in canine and feline species (reviewed in 39). Feline mucopolysaccharidosis due to α-L-iduronidase deficiency (MPS I) has been described in a domestic short-haired cat (38). A strain of Siamese cats (40) with arylsulfatase B deficiency has been reported, analogous to MPS VI (Maroteaux Lamy syndrome). More recently, Haskins and co-workers (37) have described β-glucuronidase deficiency in a dog, a condition analogous to human mucopolysaccharidosis VII. Shull and co-workers have characterized a canine model of MPS I caused by deficiency of lysosomal α-L-iduronidase in a family of Plott hounds (100, 102, 103). Affected dogs have stunted growth, severe bone disease (dysostosis multiplex), degenerative joint disease, corneal clouding, enlarged tongue, and heart disease. These features are shared by human MPS IH/S (Hurler/Scheie phenotype), for which the canine disorder is a valuable animal model.

Multiple or Overlapping Storage Disorders

The neuronal ceroid lipofuscinoses (NCL) are a group of inherited lysosomal disorders characterized by blindness, brain atrophy, dementia, and seizures (129). In humans, infantile, late infantile, and juvenile forms are recognized. Adult and atypical variant forms have been reported. The basic biochemical defect in this group of diseases is unknown. The histopathologic abnormality characteristic of all forms of ceroid lipofuscinosis is the presence of an autofluorescent lipopigment in neurons and many nonneuronal cells. Most research on the ceroid lipofuscinoses has focused on analysis of the stored lipopigment. It has been proposed that the lipopigment is derived from lipid peroxidation. A higher content of polyunsaturated fatty acids has been observed in phospholipids isolated from brains of patients with infantile ceroid lipofuscinosis (110).

Several animal models of human NCL have been described. Canine ceroid lipofuscinosis (CCL) has been reported in a colony of English setters (65), in Dalmatian dogs (22), and more recently in Border collie dogs (112). The clinical and pathologic features of CCL most closely resemble human Batten's disease (infantile NCL). Detailed studies of the retinal pigment epithelium of affected dogs have been performed (34). Additional characterization of this feature will be useful in understanding the mechanism of progressive blindness, a devastating consequence of this condition. Palmer and co-workers (78, 79) recently provided new information on the nature of the stored lipopigment in an ovine model of ceroid lipofuscinosis. Their studies indicate that the lipopigment isolated from the livers of affected sheep was largely proteinaceous. Further characterization of the protein component re-

vealed the presence of several different polypeptide species: two major bands, one at 14.8 kd and one at 3.5 kd, and a heterogeneous group of polypeptides between 9.0 and 5.0 kd, as analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. These observations led to the suggestion that the underlying defect in NCL relates to defective lysosomal proteolysis. Future studies on the ovine ceroid lipofuscinosis will undoubtedly shed more light on this enigmatic group of disorders.

THE MURINE LYSOSOMAL CHOLESTEROL STORAGE DISORDER: PROTOTYPIC USE OF AN ANIMAL MODEL

Mouse Model

The mutant cholesterol storage mouse serves as an excellent example of how studies of animal disorders can lead to significant breakthroughs in our understanding of human metabolic disorders. This particular mouse was discovered as a spontaneously occurring mutant in a colony of Balb/c mice (72). The murine disorder is transmitted as an autosomal recessive trait. Affected animals begin to display characteristic clinical signs of ataxia and tremors at 40 days. Subsequent precipitous neurologic decline is followed by death at 65–70 days. Histopathological examination shows extensive foam cell infiltration of the liver, spleen, thymus, and lymph nodes (98). Microscopic examination of affected tissues reveals cells filled with multilamellar inclusion bodies characteristic of lysosomal storage disorders. Brains of affected animals display myelin deficiency (123). Affected tissues have elevated levels of unesterified cholesterol, glycolipids, and the phospholipids sphingomyelin and bis(monoacylglycero) phosphate (85). Lipid accumulation is not seen in affected newborn animals, but storage of the lipids increases with age (85). The murine disorder is marked by deficiency of both sphingomyelinase and glucocerebrosidase and a contrasting elevation in the activities of many of the other lysosomal hydrolases (85). The chronic progressive neurologic deterioration, visceromegaly, foamy macrophage infiltration of tissues, and pattern of lipid accumulation of the murine disorder is reminiscent of a group of variant Niemann-Pick disorders (types C, D, and E), which are not associated with a primary deficiency of sphingomyelinase (10).

Investigations of the pleiotropic biochemical abnormalities that characterize the mutant Balb/c mouse have sought to explain the prominent tissue accumulation of unesterified cholesterol. When affected animals were fed a 2% cholesterol diet for four weeks, hepatomegaly and an abnormal hepatic accumulation of unesterified rather than esterified cholesterol resulted (81). Defective cellular cholesterol esterification is not caused by an inherent deficiency of acyl-CoA: cholesterol acyltransferase (ACAT), the enzyme

responsible for intracellular cholesterol esterification, since ACAT levels are normal or even elevated in cell-free extracts of affected animals (82). Intracellular esterification of exogenously derived cholesterol is also markedly impaired in cultured fibroblasts derived from homozygous affected mice and is partially deficient in cell cultures derived from heterozygous mutant animals (82). Fluorescent microscopic studies show that mutant mouse fibroblasts accumulate excessive unesterified cholesterol in their lysosomes when cultured in the presence of serum lipoproteins (P. G. Pentchev and S. C. Patel, unpublished data).

Relationship to Niemann-Pick Disease, Type C

Because of the phenotypic similarities between Type C Niemann-Pick disease and the murine cholesterol storage disorder, the ability of Niemann-Pick C (NPC) fibroblasts to process exogenously derived cholesterol was examined. An initial survey of cholesterol processing in NPC as well as in other mutant human lipid storage disorder fibroblasts led to the discovery that Niemann-Pick C disease is uniquely and specifically associated with a lesion that disrupts all phases of cellular cholesterol homeostasis (83, 84). The lesion renders the mutant fibroblasts susceptible to excessive uptake and accumulation of unesterified cholesterol derived from low density lipoprotein (LDL). Partial deficiencies in cellular cholesterol homeostasis are seen in fibroblasts derived from obligate NPC heterozygotes (67, 83, 84, 115). The deficient homeostatic responses are not caused by any inherent defects in the regulatory responses themselves (83); rather, they are a consequence of a deficient intracellular translocation of endocytosed LDL-cholesterol from lysosomes (104). The mutant cholesterol storage mouse will likely continue to aid in the study of human Niemann-Pick C disease.

USE OF ANIMAL MODELS FOR THE DEVELOPMENT OF THERAPEUTIC STRATEGIES FOR HUMAN DISORDERS

Animal models of human genetic disorders are uniquely suited for testing newer therapeutic approaches that are either impractical or difficult to conduct in humans. This suitability is particularly true for lysosomal storage disorders, and many of the well-characterized animal models of the human disorders have already been used to advantage. A number of therapeutic strategies have received considerable attention over the past decade. We review some therapeutic trials conducted in select animal analogs of human lysosomal disorders and offer our own perspectives for future studies.

Enzyme Replacement Therapy

The possibility that lysosomal storage disorders could be reversed by supplemental administration of exogenous enzymes in enzymatically deficient states has received attention since the original conceptual formulation of lysosomal storage disorders (41). The theoretical basis for such direct "gene-product" replacement stems from the observation that lysosomes are in contact with the extracellular space via the continual process of endocytosis. The administration of exogenous enzyme to a cell can lead to its endocytic uptake and subsequent fusion with lysosomes, where its enhanced presence may mobilize the hydrolysis of accumulated substrate. Numerous trials using such an approach have been conducted in humans and animal models. In general, such studies have met with mixed success. In spite of several examples demonstrating both the uptake of administered enzymes into various tissues and the effective subsequent clearance of accumulated substrate, the practical utility of such an approach to human disease has yet to be realized.

A major hurdle of enzyme replacement therapy is in targeting administered enzyme to the appropriate organ and cell. Lysosomal enzymes are glycoproteins with *N*-asparagine-linked "complex" carbohydrate residues. Their cellular uptake is often mediated by cell surface receptors that recognize specific terminal glycosyl residues. Uptake by the liver may be mediated either by specific receptors on Kupffer cells that recognize terminal mannose or *N*-acetyl glucosamine residues (90) or by receptors on hepatocytes that interact with terminal galactose residues (74). These receptor-mediated interactions must be exploited to direct exogenously administered enzymes to the appropriate storage cells. Extensive studies on β-glucuronidase–deficient mice by Thorpe and co-workers (113) established that intravenously administered bovine β-glucuronidase was rapidly cleared from the murine circulation ($t_{1/2}$ $\sim$ 3 min) and taken up almost exclusively by hepatic lysosomes. Similar observations were made by Rattazzi et al (90) with β-hexosaminidase infusion in cats with G_{M2} gangliosidosis. Hepatic hexosaminidase levels that were markedly attenuated prior to enzyme infusion were increased to 30% of normal. This increase was associated with a marked reduction of hepatic G_{M2} ganglioside and globoside levels. Transfer of hexosaminidase into the brain, however, was precluded because of the blood-brain barrier. To increase delivery of the enzyme into the brain, hexosaminidase was given intravenously concurrently with mannosyl-rich *Saccharomyces cerevisiae* mannans (to inhibit hepatic glycosyl receptor-mediated uptake). This administration was combined with intracarotid microembolization of small volumes of oxygen to open the blood-brain barrier (91). Modest increases in brain β-hexoasaminidase levels were obtained (10% of normal), but there was no corresponding reduction in brain ganglioside G_{M2} storage.

In the future, recombinant DNA technology will allow the production of

adequate quantities of lysosomal enzymes to further pursue enzyme replacement trials. With appropriate modifications of the genetically engineered enzyme, it may be possible to deliver the proteins to specific target sites of interest in large quantities. Animal analogs of human lysosomal storage diseases clearly play a vital role in such trials. Traversing the blood-brain barrier, however, remains a formidable challenge for direct gene product replacement. A possible alternative lies in the direct use of donor cells with a normal complement of lysosomal enzymes that may migrate to protected sites such as the nervous system to provide a repository of enzyme-producing cells. Allogenic tissue transplantation may offer such an alternative.

Allogenic Tissue Transplantation

BONE MARROW TRANSPLANTATION (BMT) The use of bone marrow transplantation in the treatment of inherited metabolic diseases has increased recently. Bone marrow transplantation has now been attempted in a number of human lysosomal storage diseases with variable clinical improvement (66). In general, improvement has primarily been in extraneural manifestations such as visceromegaly and corneal and joint involvement. In some cases neural manifestations have also benefited. The mechanism for clinical and metabolic improvement may represent direct replacement of metabolically deficient recipient cells in affected tissues with enzymatically competent donor cells as well as enzyme transfer from donor cells to the circulation and/or recipient cells (101). Recent studies on bone marrow transplantation in several animal analogs of human lysosomal storage diseases have shown great promise.

Bone marrow transplantation substantially prolongs survival in the twitcher mouse (127). Ichioka et al (50) found that transplantation of enzymatically normal congenic bone marrow restored galactosylceramidase activity to normal levels in brain and liver of the twitcher mouse from pretreatment levels that were 8% of control values. More importantly, psychosine, the presumed neurotoxic factor in Krabbe's disease (111), showed a sustained decrease to 30–35% of control values in the brain of treated twitcher mice. In the peripheral nerve, there was an initial decline of psychosine but levels gradually returned to pretreatment levels in 100-day-old animals. In the kidney, galactosylceramidase activity could be restored to only 20–30% of control levels.

In a separate study, Suzuki and co-workers (107) also found an increase in galactosylceramidase activity in the visceral organs and the central (CNS) as well as the peripheral nervous systems (PNS) of BMT–treated twitcher mice. Positive morphologic effects of BMT were most apparent in animals that showed prolonged survival. Morphologic examination showed that the white matter of treated mice had been infiltrated with foamy macrophages that were believed to be derived from donor cells with normal levels of β-galactosidase.

These cells had presumably crossed the blood-brain barrier of the affected animals and migrated into the CNS. Globoid cells disappeared from the white matter of these treated animals, and additional changes indicated remyelination. However, inclusions were still found in the oligodendrocytes of BMT-treated twitchers at 100 days of age, which suggests that the enzymatic deficiency in these cells had not been completely corrected. These observations are particularly important for future human trials with BMT, since at least some reversal of the white matter pathology as well as prolonged improvement might be expected in conditions such as Krabbe's disease.

In a recent study on BMT in cats with MPS VI, Wenger and co-workers (124) noted stable engraftment of transplanted bone marrow associated with conversion of very low leukocyte arylsulfatase levels to the normal range and improvement in clinical symptoms. In the converse experiment, bone marrow from an affected cat was transplanted into a normal cat. Leukocyte arylsulfatase activity dropped to very low levels in the recipient, but there were no clinical changes and mucopolysaccharide catabolism remained normal. It was suggested that non-blood-derived cells in the recipient animal were capable of maintaining normal mucopolysaccharide degradation.

The results of bone marrow transplantation in canine mucopolysaccharidosis are equally noteworthy. In a recent study of three long-term survivors of BMT, Shull et al (101) found small but significant levels (1–3% of donor values) of α-L-iduronidase activity in the brains of treated animals. Higher levels of α-L-iduronidase activity were measured in the cerebrospinal fluid (7–15% of donor values). These findings were associated with significant reductions in stored glycosaminoglycans in the brain as well as with clinical and neuropathologic signs of improvement in the treated animals (99).

Enzyme transfer between different cell types may be an important mechanism that mediates some of the improvement seen with BMT. Transfer of lysosomal enzymes between different cell types in tissue culture and effective clearance of stored substrate in the enzyme-deficient cells have been documented in several cases (1, 77). Although varied in outcome, the remarkable improvement with BMT documented in several lysosomal storage disorders in humans (23, 42, 48) and in animal analogs of the human disorders (50, 101, 107, 124, 127) should encourage further studies.

ORGAN AND TISSUE TRANSPLANTATION Allotransplantation of an organ or tissue in which a missing lysosomal enzyme is normally synthesized has been attempted in several lysosomal storage diseases. Renal transplantation is often required for end-stage renal insufficiency in Fabry's disease. However, attempts to use renal allografts to correct α-galactosidase deficiency in nonrenal sites such as vascular endothelium and in neural tissues have not lead to sustained improvement (105). Trials of spleen and kidney transplantation in

Gaucher's disease were unsuccessful (9). Recently, Adolfini & Brown (2) adopted a more novel approach by using transplantation of normal amnion cells into patients with Hunter and Hurler's disease. Amnion cells were chosen because they synthesize large quantities of lysosomal enzymes, do not express histocompatibility antigens, and did not undergo rejection in trials on normal volunteers. "Modest clinical improvement" was described in the treated patients. Howell and co-workers (46) applied a similar approach by using multiple implantation of normal amnion into cattle with generalized glycogenosis type II. Their results were disappointing because of a high incidence of rejection and no demonstrable improvement in clinical symptoms. Overall, the experience with allotransplantation in animal analogs of human lysosomal diseases has thus far been limited, but as newer and more potent immunosuppressants become available, tissue transplantation will be an important therapeutic strategy for which animal models could play a useful role.

Gene Therapy

Should it become technically feasible, the ideal treatment for inherited metabolic disorders would be gene therapy. In principle such treatment would entail not only insertion of DNA containing the normal gene into the defective host genome but also appropriate management of its expression in host tissues. At the present time, retroviruses appear to be the preferred vectors for introducing exogenous genes into cells in vivo and in vitro (17) because of the high efficiency of expression of the integrated viral genes and the integration of the viral gene as a single copy of DNA at a single random site. With the development of new vectors that contain all the regulatory signals necessary to produce controlled expression in targeted cells, retroviruses may eventually offer the best means for gene therapy. The use of animal models will be a vital link in such studies.

Future Therapeutic Applications

Animal models of human lysosomal storage disorders have played a central role not only in unraveling the molecular pathology of specific lysosomal deficiencies but also in the testing of various therapeutic strategies. Available animal analogs of inherited metabolic disorders in humans have thus far been limited to spontaneously occurring mutants. The ability to construct transgenic animals, however, now allows the development of mutants with predetermined genetic defects (51). For example, initial studies (68) have been conducted on the development of a murine analog of the human disorder, Lesch-Nyhan syndrome, which is caused by hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency. Embryonic mouse stem cells were mutagenized, and clones that had lost the ability to produce HPRT were

selected. Transgenic mice were produced from these clones and found to be HPRT deficient. A surprising finding in these studies was that although the mice were HPRT deficient, they did not manifest the neurologic damage characteristically seen in the Lesch-Nyhan syndrome. This lack of expression is most likely due to the differences in purine metabolism between humans and mice.

SUMMARY AND CONCLUSIONS

In this article we have described studies using animals with genetic lysosomal defects with particular emphasis on mutant disorders that have contributed toward understanding of pathogenesis and/or management of analogous human conditions. The development of newer strategies in the production and use of animal models will greatly expand the contribution of this resource to biology and medicine.

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