Nephropathic cystinosis was first described in the early 1900s in a 21-mo-old boy who died of progressive anorexia; two siblings had previously died in infancy under similar circumstances (1). By meticulous observations and analyses, it became clear that abnormal cystine accumulation was characteristic of this autosomal recessive disease (2-4). Although some considered it to be a severe form of cystinuria, cystinosis was clearly distinguished from cystinuria by Bickel’s excellent clinical and biochemical observations (5). Clinically, untreated cystinosis patients would suffer renal tubular Fanconi syndrome, with hypophosphatemic rickets, hypokalemia, polyuria, polydypsia, dehydration, acidosis, and growth retardation followed by end-stage renal disease (ESRD) and death at approximately 10 yr of age (6,7). Shortly after the distinctive clinical aspects of cystinosis were defined, the intracellular location of cystine storage was determined. In the late 1960s, Schneider et al. (8), Schulman et al. (9), and Patrick and Lake (10) performed elegant biochemical studies to demonstrate that cystinosis cells stored cystine within the lysosome, an organelle discovered only two decades before (11). Subsequent work showed that cystine was transported out of normal lysosomes by a carrier system that exhibited countertransport, saturation kinetics, and stereospecificity (12,13); this system was defective in cystinosis lysosomes (12,14,15) and displayed a gene-dosage effect in heterozygotes for cystinosis (16).

The major therapeutic breakthrough for cystinosis children came with the discovery that cysteamine (β-mercaptoethylamine) could deplete cells of cystine (17) by combining with cystine to produce cysteine and cysteine-cysteamine mixed disulfide, which exits cystinotic lysosomes via a functional lysine carrier (18). This finding made meaningful therapy possible, and cysteamine was subsequently shown to retard renal deterioration and enhance growth in cystinosis children (19). Patients treated early (i.e., before 1 yr of age) and diligently with oral cysteamine are expected to preserve their kidney function for several decades and might even avoid requiring a renal allograft altogether (20). In fact, oral cysteamine therapy has become the treatment of choice for cystinosis (6) and was approved by the United States Food and Drug Administration on August 15, 1994, for the treatment of pre-transplant cystinosis patients. At the same time, eyedrops containing cysteamine (0.5%) were shown to dissolve the corneal crystals, which cause a painful photophobia and occasional epithelial erosions (21-23). The crystals are pathogenic for cystinosis and can be identified by an experienced ophthalmologist as early as 1 yr of age (24).

The era of molecular biology has brought with it an understanding of the genetic basis of cystinosis. In the mid 1990s, the cystinosis gene was mapped to chromosome 17p (25); in 1998, the gene C7NS, coding for a lysosomal transport protein named cystinosin, was isolated (26). A 57,257-bp deletion (27) was found to be responsible for approximately half of Northern European and North American cystinosis patients (26,28); this deletion is easily detected by a multiplex PCR amplification assay (29). Cystinosis occurs in all ethnic groups, and 56 different mutations have been described to date, including promoter, missense, nonsense, deletion, insertion, and splice-site mutations (26,28,30–36). The allelic disorders, intermediate cystinosis (with late-onset renal disease) and ocular cystinosis (limited to corneal involvement), are much more rare and result from the combination of one severe (nephropathic) mutation and one mild mutation in C7NS (30,33).

Isolation of the cystinosis gene has confirmed previous findings concerning cystinosin and has revealed new facts about integral lysosomal membrane proteins with transport function. Cystinosin contains 367 amino acids, including seven transmembrane regions, eight potential N-linked glycosylation sites, and a lysosomal-targeting motif at the carboxy terminus (26). A second targeting motif necessary for proper placement of cystinosin in the lysosomal membrane was later demonstrated using green fluorescent protein (GFP)–tagged normal and mutant cystinosin fusion proteins (37). Other experiments showed that normal cystinosin localized in lysosomes and colocalized with a lysosomal marker protein, LAMP-2 (37). Recent studies have demonstrated that cystinosin acts as a proton-driven lysosomal cystine transporter, which confirms previous findings (38). Kalatzis et al. (39) deleted the lysosomal targeting motif of cystinosin at its carboxy terminus, causing misrouting to the plasma membrane. Incorporation of the mutant but still functional cystinosin protein into the plasma membrane allowed cystine transport studies to be performed at the whole-cell level. These studies showed pH dependence, substrate specificity, and kinetic data that confirmed previous results obtained using crude lysosome–rich granular fractions (12-14). Other investigators incubated fibroblasts from cystinosis patients carrying a nonsense mutation with gentamicin, which can cause read-through of nonsense mutations; this led to correction of the mutation and depletion...
of intracellular cystine (40). In addition, GFP fused to the N-terminal and C-terminal ends of cystinosin verified the lysosomal location of this protein (40), again confirming previous work with newer and more sophisticated techniques.

In this issue of the JASN, Haq et al. (41) report that they have developed a powerful antibody directed against the carboxy terminal portion of cystinosin. Although endogenous levels of cystinosin in normal and cystinotic cells in culture were not examined, transfected COS-7 cells expressing wild-type cystinosin showed an antibody reaction whereas cells expressing mutant cystinosin did not. The antibody also correctly identified the lysosomal location of cystinosin and its presence in the renal tubular cells of normal kidneys. Indeed, the signal was missing from the kidneys of cystinosis patients homozygous for the common 57-kb deletion.

This antibody represents an important advance for future investigations into cystinosis because it can help answer a litany of questions. If missense mutations produce cystinosin proteins that remain antigenic, the antibody can be used to follow the intracellular location of the abnormal proteins. The mutations characterizing ocular (33) and intermediate (30) cystinosis will be of special interest. The antibody can be used to ascertain the expression level of the cystinosin protein in normal human tissues, as well as in ocular cystinosis tissues such as the kidney, if they become available. It may also crossreact with murine cystinosin and could prove useful in mouse studies, including those involving a knockout mouse. Pulse-chase experiments using metabolic labeling and polyacrylamide gel electrophoresis-autoradiography will determine the extent and time course of glycosylation and processing of cystinosin. Finally, the antibody may help answer the question of why I-cells store cystine (42,43). I-cells, or mucolipidosis-II cells, have deficient mannose-6-phosphate targeting of enzymes to lysosomes (44). One hypothesis is that a deficient contingent of lysosomal enzymes could cause cystinosin to be improperly processed and therefore dysfunctional.

Cystinosis has taught us volumes about lysosomal membrane transport and its genes and will reveal more about trafficking and processing of integral lysosomal membrane proteins. It may even inform us of the various subsets of lysosomes that must exist and of how they form. Cystinosin antibodies will assist in these academic endeavors; however, for the children and adults who cope daily with a devastating systemic disease, the main goal is to endure and prevail. Unlike those in previous generations who succumbed to dehydration or uremia, today’s cystinosis patients have cysteamine as a disease-altering therapy. This treatment should be universally embraced for pretransplant patients and strongly considered for posttransplant use. In addition, the medical profession must work diligently to promote early treatment and earlier diagnosis, complete with attempts at newborn screening.

References


