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An animal model of glomerular light-chain-associated amyloidogenesis depicts the crucial role of lysosomes

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***In vitro* and *ex vivo* studies have elucidated the step-by-step process whereby some physicochemically abnormal light chains are processed by mesangial cells to form amyloid fibrils. Although crucial steps in the cascade of events have been determined, these findings have not been reproduced *in vivo*. This has led to some doubts as to the significance and clinical application of the information that has been deciphered. Here, we developed an animal model which uses mice injected with amyloidogenic light chains purified from the urine of patients with biopsy-proven, light-chain-associated glomerular amyloidosis which validated *in vitro*/*ex vivo* findings. This animal model showed internalization of the light chains utilizing caveolae followed by trafficking to the mature lysosomal compartment where fibrils were formed. This model permits evaluation of mesangial amyloidogenesis for prolonged periods of time, is potentially useful to test maneuvers to modulate events that take place, and can be used to design novel therapeutic interventions.**

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In 1927, Smetana[1] pointed out the important role of the reticuloendothelial system in amyloidosis. *In vitro* models were used since the 1960s to elucidate mechanisms involved in the process of amyloidogenesis. In the early 1960s and 70s, Shirahama and Cohen conducted seminal research in the pathogenesis of AA-amyloidosis using macrophages where they indicated that the lysosomes played an important role in amyloidogenesis [2–5] and studied glomerular changes in human primary and secondary amyloidosis and experimental AA-amyloidosis (in rabbits).[6] Interestingly, the authors noted a distinct population of mesangial cells with a macrophage phenotype that they incorrectly interpreted at the time as representing degenerating mesangial cells. Gueft, at the time a post-doctoral research fellow, and Guidoni in 1963 stated: "We suggest that the amyloid fibrils are formed at the moment that a histiocyte, etc, delivers the precursor (protein) to the outside"[2] supporting the important role of macrophages or facultative macrophages in the process of amyloid formation and suggesting that amyloid fibrils are released at the surface of the cells engaged in their genesis. These concepts at the time were highly innovative and provocative.

Studies by other investigators 30–40 years later further highlighted the important role played by lysosomes in macrophages in the pathogenesis of AA-amyloidosis, but none of these studies specifically addressed the pathogenesis of renal amyloidosis.[7,8] These studies concluded that amyloid formation occurred intracellularly supporting other early studies on the subject performed using immunofluorescence and electron microscopy.[9] Whether amyloid is ever located intracytoplasmically before it is extruded from the cells during amyloidogenesis has been a source of debate. For years, the definition of amyloid indicated that it was an extracellular material; however, such definition has been changed to indicate that it may also be found intracellularly, based on experimental data obtained from studies of macrophages engaged in the formation of fibrils in AA-amyloidosis.

In 1987, Cohen and Connors[10] summarized the knowledge that had been collected on the subject of amyloidogenesis in a comprehensive manuscript which addressed biochemical aspects and pathogenesis. In 1999, Solomon *et al.*[11] created a transgenic mouse model of AA-amyloidosis. These animals developed systemic amyloidosis and died of renal failure.

However, no detailed studies of renal amyloidogenesis were performed using this experimental platform.

Tagouri *et al.*[12] were the first to demonstrate *in vitro* amyloid formation by mesangial cells grown in monolayers and on Matrigel incubated with monoclonal light-chains (LCs) obtained from the urine of patients with renal biopsy-proven glomerular light chain derived (AL)-amyloidosis. Additional work performed later addressed the formation of AL-amyloid in glomeruli in an *ex vivo* experimental platform,[13] perfusing monoclonal LCs through the renal artery in mice kidneys maintained physiologically intact. The *ex vivo* platform could only be utilized up to 72 h after the kidney is removed from its normal blood supply, as the kidney could not be maintained physiologically intact for a more extended period of time.

RESULTS

LCs purified from the urine of patients with AL-amyloidosis (Figure 1) and renal biopsy-proven involvement injected in the penile vein of mice were successfully delivered to the mesangium (Figure 2). Not all glomeruli received the same amount of LCs at any given time frame, but the injected LCs were demonstrated consistently in the mesangium in the majority of the glomeruli in both kidneys. The amount of LCs in different mesangial areas varied but was similar at 1 and 2 weeks post initial LC injection (Figure 2). This finding was important as the delivery of the LCs to the kidneys in acceptable quantities for prolonged periods of time, so that the process of amyloidogenesis could be studied, has been a challenge in previous experimental *in vivo* platforms. The LCs were more concentrated in the mesangial areas at two weeks post initial injection (Figure 2). There were striking differences in glomeruli from mice injected with myeloma cast nephropathy (MCN), light chain deposition disease (LCDD), and amyloidogenic light chains at 2 weeks post initial injection (Figure 3).

In this *in vivo* model, amyloid deposits were identified in the glomerular mesangium and in arterioles and small-size arteries (Figure 3j–m). The entire process of amyloid formation in the mesangium could be followed sequentially and documented. In contrast, in mice injected with LCDD-LCs, initial mesangial expansion with hypercellularity and later increased matrix was observed (Figure 3d–f), and glomeruli in those injected with MCN-LCs (Figure 3a–c) were normal. No amyloid was seen on Congo red or Thioflavin T stains in the renal parenchyma of mice injected with LCDD (Figure 3g and h) or MCN-LCs (Figure 3b and c).

Following internalization, the amyloid-forming (but not the LCDD-LCs which were catabolized in endosomes or the MCN-LCs which did not interact with mesangial cells) LCs reached the mature lysosomal compartment; this process took ~2 h, with some variation depending on the LC tested. The longest component of the sequence of events was the lysosomal processing of the LCs to form the amyloid fibrils which took 8–10 h. The overall speed of amyloid formation and the amount produced varied among LCs utilized in the experiments. The identity of the fibrillary material as amyloid was established based on the staining characteristics of the material (salmon pink staining with Congo red stain (Figure 3k) and subsequent apple-green birefringence upon polarization (Figure 3l), and thioflavin T fluorescence (Figure 3m) and, most importantly, the ultrastructural features of the fibrils seen by transmission electron microscopy supported by the scanning electron microscopic appearance. The fibrils were randomly arranged, measured 7–14 nm (mean = 11 nm) in diameter, and did not show branching.

Approximately 60 min after incubation with the amyloidogenic LCs (depending on the LC), mesangial cells began to

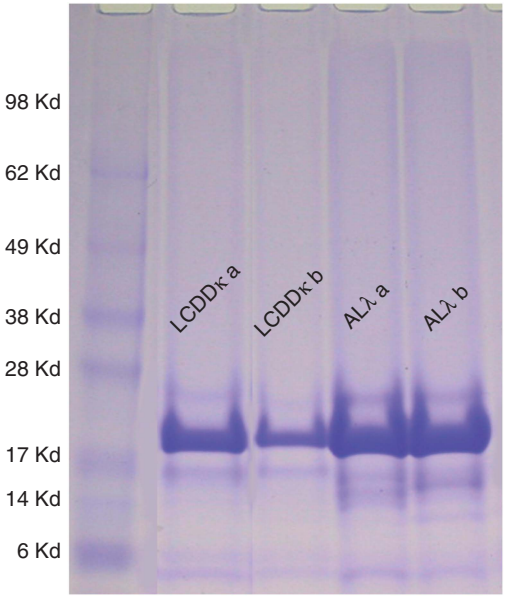

Figure 1 | SDS-polyacrylamide gel electrophoresis (PAGE) of purified light chains. Two of the amyloidogenic and two light chain deposition disease (LCDD) light chains injected in mice are shown as single bands at about 20–25 Kd.

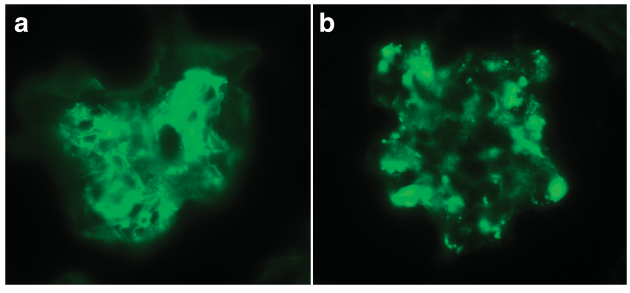

Figure 2 | Monotypical light chains in glomeruli. (**a**, **b**) ×500 (**a**) and ×500 (**b**), direct immunofluorescence for kappa and lambda light chains, respectively; fluorescein isothiocyanate, marker dye. One (**a**) and two (**b**) weeks post initiation of injection of amyloidogenic light chains. Note deposition of light chains in mesangial areas at 1 and 2 weeks. At 2 weeks, the deposited light chains are more concentrated in mesangial areas.