American Journal of Pathology, Vol. 140, No. 3, March 1992

Induction in Mice of Human Light-chain–associated Amyloidosis

Alan Solomon,* Deborah T. Weiss,* and Mark B. Pepys†

From the Department of Medicine, Human Immunology and Cancer Program, University of Tennessee Medical Center/Graduate School of Medicine, Knoxville, Tennessee; and the Department of Medicine,† Immunological Medicine Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom*

Primary (idiopathic) or multiple myeloma-associated amyloidosis is characterized by the deposition in tissue of monoclonal light chains or light-chain fragments (AL amyloidosis). In contrast to other types of amyloidosis, information regarding the pathogenesis of light-chain–related amyloid has heretofore been limited due to the lack of a suitable* in vivo *model. The authors report the successful experimental induction of human AL amyloid deposits. The repeated injection into mice of Bence Jones proteins obtained from two patients with AL amyloidosis produced the histopathologic lesions characteristic of this disease. Partial dehydration of animals before protein injection resulted in the acceleration of amyloid formation. The human proteins were deposited as amyloid within the mouse renal blood vessel walls and parenchymal tissue, as well as in other organs. The deposits were Congo red-positive, exhibited green birefringence, and had a fibrillar ultrastructure. As evidenced immunohistochemically, the experimentally induced amyloid deposits consisted of the injected human light chains, and in addition, contained mouse amyloid P component (AP); mouse immunoglobulin (Ig) or inflammatory-associated amyloid A protein was not detected. Extraction and characterization of the amyloid deposits found within the mouse kidney revealed the presence of a predominately intact human light polypeptide chain. Mice injected in identical manner with a non–amyloid-associated Bence Jones protein had no or only rare amyloid deposits. The experimental mouse model provides a means to ascertain the amyloidogenic potential of human monoclonal light chains and to study further the pathogenesis of AL amyloidosis. (Am J Pathol 1992, 140:629–637)

In 1964, Osserman and colleagues[1–3] predicted that monoclonal immunoglobulins (Igs) found in patients with plasma cell dyscrasias could be implicated in amyloidogenesis. This hypothesis was subsequently confirmed by Glenner et al,[4] who demonstrated the seminal role of the light polypeptide chain in the pathogenesis of primary or multiple myeloma-associated amyloidosis, i.e., AL amyloidosis. This disease is distinguished by deposition in tissue of monoclonal light chains or light-chain-related fragments, and like in all types of amyloidosis, these deposits have characteristic tinctorial and ultrastructural features, including green birefringence after Congo red staining and 8- to 10-nm nonbranching fibrils evidenced by polarizing and electron microscopy, respectively.[5,6] Additionally, a minor but universal constituent of all amyloid deposits, both in human and animals, is the amyloid P component (AP), a nonfibrillar glycoprotein of the pentraxin family[7] identical to a normal circulating protein designated serum amyloid P component (SAP).[8,9]

The fact that the mere presence of a serum or urinary monoclonal Ig does not invariably result in amyloid formation implies that either certain types of light chains are inherently amyloidogenic or that other pathophysiologic factors are responsible for the development of amyloid. The availability of an *in vivo* model for AL amyloidosis would thus provide a means to study further the pathogenic role of protein, as well as host factors, in this disease. Although it is possible to induce another form of amyloidosis experimentally, e.g., through injection of mice with casein or endotoxin,[5] the type of amyloid protein deposited is not Ig-related. Rather, this protein, designated amyloid A, represents a degradation product of an acute-phase serum protein (serum amyloid A or SAA) and is the principal constituent of the amyloid associated with chronic infectious or inflammatory disease, i.e., AA amyloidosis.[6–10]

Supported in part by U.S. Public Health Research Grant CA 10056 from the National Cancer Institute, the Ina M. Barger Memorial Grant for Cancer Research (IM-430) from the American Cancer Society, the Stein Cancer Research Fund, and the United Kingdom Medical Research Council.

Accepted for publication September 20, 1991.

Address reprint requests to Dr. Alan Solomon, University of Tennessee Medical Center at Knoxville, 1924 Alcoa Highway, Knoxville, TN 37920.

In an effort to elucidate the protein and/or host factors essential to the development of AL-type amyloid, we have used the experimental model first described in 1976 by Koss, Pirani, and Osserman.[11] These investigators found that a single intraperitoneal injection into mice of a human Bence Jones protein produced the renal lesions characteristic of myeloma (cast) nephropathy. We have confirmed this finding in a study of 40 different Bence Jones proteins whereby we determined that the injection of certain Bence Jones proteins into mice resulted not only in the renal deposition of the human light chains as casts, but also as crystals or basement-membrane precipitates.[12] In addition, the form and location of Bence Jones protein deposition noted experimentally was comparable to that found clinically. In contrast to these experiments, the induction of amyloid in mice required multiple injections of Bence Jones proteins obtained from patients with AL amyloidosis.[12–13] We report in detail the conditions that resulted in the experimental *in vivo* deposition of human light chains as amyloid, the characterization of the light chains extracted from the induced amyloid, and the demonstration that such deposits also contained mouse amyloid P component.

Methods and Materials

Proteins

Monoclonal light chains, i.e., Bence Jones proteins, were isolated by preparative zone (block) electrophoresis[11] from urine specimens obtained from patients with AL amyloidosis or multiple myeloma. The purity of the protein preparations was established by electrophoresis in agarose gels and by SDS/PAGE. The proteins were analyzed immunochemically using our rabbit anti-human κ and λ light-chain isotype-specific antisera as well as anti-V_κ- or anti-V_λ-subgroup-specific antisera.[14] The V_L-subgroup nature of the Bence Jones proteins determined serologically was confirmed by amino acid sequence analyses.

Experimental Protocol

Six-week-old C3H/HEJ mice (weighing 15–20 g) were injected intraperitoneally with reconstituted, sterile filtered solutions of Bence Jones protein using a 3-ml luer-lock Monoject syringe (Sherwood Medical, St. Louis, MO) and 25-gauge needle. The protein was dissolved in 1 ml of a sterile physiologic buffer solution, pH 7.1, prepared by adding 0.5 ml of 4% sodium bicarbonate (Neut®, Abbott Labs., North Chicago, IL) to 10 ml of preservative-free 0.9% sodium chloride (Abbott Labs, North Chicago, IL) and centrifuged 3580 X g for 15 minutes. To remove insoluble protein and to sterilize the solution, the supernatant was passed through wetted 0.45-μm and 0.22-μm filters (Millipore Corp., Bedford, MA). Maximum recovery of protein was ensured by washing the filters with an additional 0.5 ml of buffer. A sufficient amount of Bence Jones protein was dissolved initially to yield, after centrifugation and filtration, the requisite final concentration of protein, as determined by a modification of the Folin-Ciocalteau method.[14] The desired volume of fluid injected was ~1.5 ml (maximum volume, 3 ml) and contained up to 300 mg of protein. After the designated period of protein administration, the mice were sacrificed by cervical dislocation and the organs were removed. Tissue was processed in two ways: for light microscopy and immunohistochemistry, sections were either placed in 10% buffered formalin and embedded in paraffin or were embedded in optimum cutting temperature (OCT) medium (Miles Inc., Elkhart, IN) and snapfrozen in an isopentane bath chilled with liquid nitrogen; for electron microscopy, sections were placed in 2.5% glutaraldehyde-0.05 M sodium cacodylate buffer, pH 7.2, and after 1 to 2 hours, were transferred to the cacodylate buffer and embedded in Epon®.

Histopathology

For light microscopy, 4- to 6-μm tissue sections were cut and stained routinely with hematoxylin-eosin; for detection of amyloid, the sections were treated with a freshly prepared alkaline Congo-red solution and examined under polarized light using a Leitz filter polarizer with a gypsum plate and a filter analyzer. For electron microscopy, Epon®-embedded sections were examined with a Zeiss 9S transmission electron microscope and photographed.

Immunohistochemistry

Six-μm paraffin-embedded tissue sections were cut on a microtome, mounted on poly-L-lysine-coated slides, placed in a 37°C incubator overnight, and deparaffinized. Four-μm snapfrozen tissue sections were cut in a cryostat, placed on poly-L-lysine-coated slides, and air-dried overnight. Immunostaining was performed as described previously.[15]

For detection of mouse AP, air-dried cryostat sections (5 μm) of snapfrozen tissue were wetted and blocked by incubation at room temperature for 10 minutes with 10% normal rabbit serum in 10 mM Tris-buffered saline (TBS). The sections were exposed for 60 minutes to a 1:200 dilution of a specific sheep anti-mouse SAP antiserum[16] (this dilution of the anti-SAP antiserum had been previously demonstrated to give optimal specific staining of amyloid deposits, without any nonspecific background, in titration experiments on spleen from mice with casein-induced amyloidosis). As controls for specificity of stain-

ing, other sections were incubated with 1:200 dilutions of normal sheep serum and the sheep anti-mouse SAP antiserum, which had been absorbed with pure mouse SAP to remove all anti-SAP activity. The sections were washed twice for 5 minutes with TBS containing 0.005% Triton X-100 (TBS-T), followed by TBS alone for a further 5 minutes, and finally stained with a 1:80 dilution of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-sheep IgG. After a 30-minute incubation with the FITC conjugate, the sections were washed twice with TBS-T and once with TBS before being mounted in Citifluor (Citifluor Ltd., London, United Kingdom).

Amyloid Extraction

The methods employed for extraction of amyloid from tissue were as described by Pras et al.[17] Mouse kidney was homogenized with 1.5 ml of cold saline in an ice bath using an Omni-Mixer (Omni International, Inc., Waterbury, CT). The extract was centrifuged at 10,000 rpm for 30 minutes at 4°C and the pellet re-extracted twice more with cold saline, once with 0.1 M Tris-sodium citrate-buffered saline, pH 8.0, and then again with saline until the A_{280} of the supernatant was < 0.10. The resultant 10,000-rpm pellet was homogenized with cold distilled water and the extract centrifuged at 35,000 rpm for 3 hours at 4°C. The pellet obtained from the water extract was then lyophilized.

Immunoblotting

The immunodetection of light chains and related components (Bence Jones protein and extracted amyloid) was performed by Western blotting; SDS/PAGE was done under reducing conditions using 20% homogeneous gels (Phastgels, LKB Pharmacia, Piscataway, NJ). Proteins were transferred by electroblotting onto PVDF membranes (Millipore Corp, Bedford, MA). After blocking nonspecific protein-binding sites with a 1% nonfat dry milk solution, the membranes were immunostained with mouse anti-human light-chain antibodies. Bound antibody was detected using an avidin-biotin complex (ABC, Vector Laboratories, Burlingame, CA) and alkaline phosphatase as the enzyme marker.

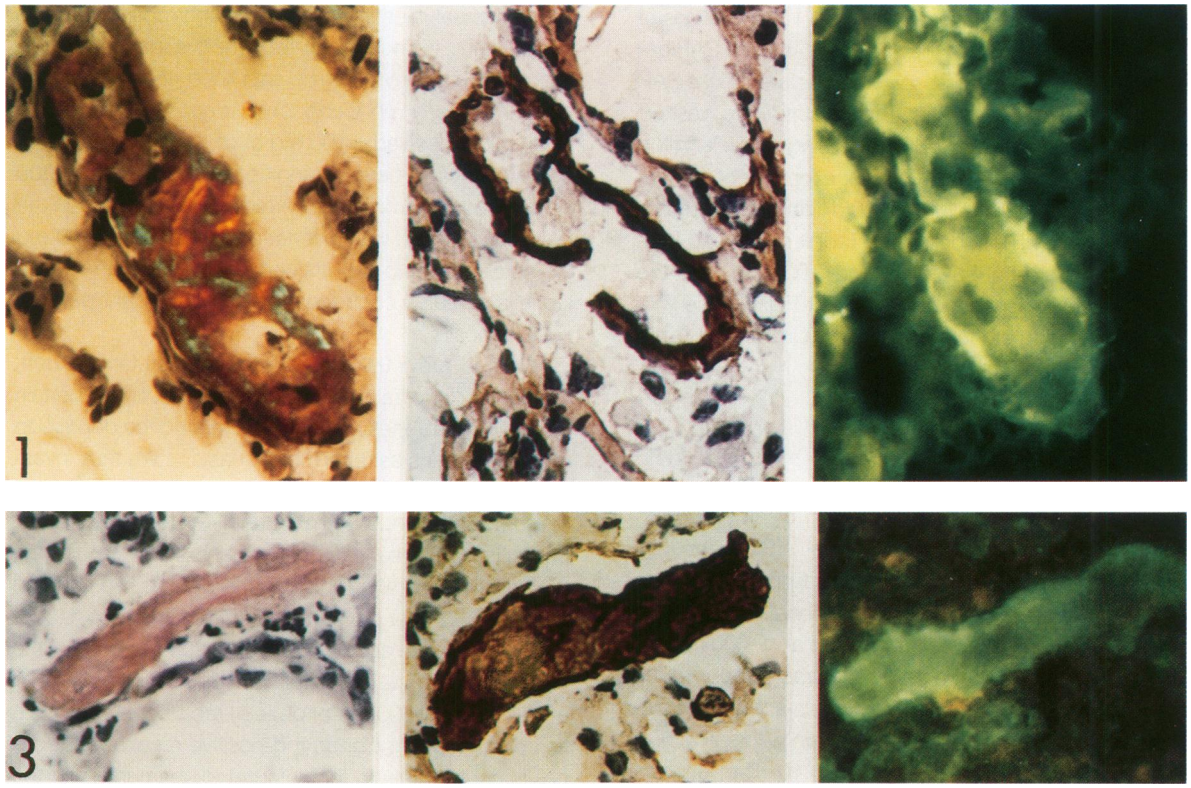
Results

Based on our finding that human λ chains of a particular V_λ subgroup—$V_{\lambda VI}$—are preferentially associated with AL-type amyloid,[18–20] we initially selected for study in the mouse model one such Bence Jones protein. Attempts to produce amyloid lesions in mice by single 300-mg intraperitoneal injections of this λVI light chain were unsuccessful, i.e., no Congo-red positive deposits were found in the kidneys of mice sacrificed 48 hours postinjection. Similarly, no Congophilic deposits were found when mice received ~100 mg of protein each of 5 consecutive days for periods ranging from 2 to 5 weeks (900 to 2200 mg of administered protein). However, when the period of injection was extended to 12 weeks (5300 mg of protein), Congo-red–stained sections of the mouse kidneys revealed occasional green birefringent perivascular deposits.

We had previously found in other short-term experiments that dehydration of mice before injection of certain human Bence Jones proteins accelerated the deposition of these proteins as renal tubular casts.[21] We thus investigated if water deprivation would also enhance in mice the formation of human light-chain–related amyloid deposits. In the first series of experiments, mice were dehydrated by temporarily removing the water bottle from the cage for a 48-hour period before protein injection. Under this condition, the administration of a 200-mg dose of the λVI Bence Jones protein given three times over an 8-week period (600 mg of protein) resulted in the formation of birefringent Congo-red positive renovascular deposits comparable to those found experimentally in the nondehydrated mouse that received ~5 gm of the human light chain.

Based on these results, we increased in subsequent experiments the total amount of protein injected to ~2 gm. Mice were dehydrated for 24 hours before each 200-mg injection of the λVI Bence Jones protein given 10 times over an 8-week period. Extensive renovascular amyloid was found, as evidenced in Congo-red–stained sections in which green birefringent deposits were present within blood vessel walls (Figure 1, left), as well as interstitially. These Congophilic deposits contained the injected human light chain, as demonstrated immunohistochemically using a monospecific anti-human λ-light-chain antiserum (Figure 1, middle). The experimentally induced amyloid deposits did not react with anti-mouse Ig or anti-mouse amyloid A antisera. Under the same experimental conditions, no renal amyloid deposits were found in mice injected with a Bence Jones protein obtained from a patient who did not have amyloidosis. No anti-human light-chain antibodies were detected in the sera of mice injected with either Bence Jones protein.

To determine if the human light-chain amyloid deposits induced in the mouse contained AP, kidney sections were examined immunohistochemically for its presence. Using a specific anti-mouse SAP antiserum, bright fluorescence was noted in vascular structures corresponding precisely in location to those that were Congo-red positive and displayed green birefringence (Figure 1, right). Sections stained either with anti-mouse SAP an-



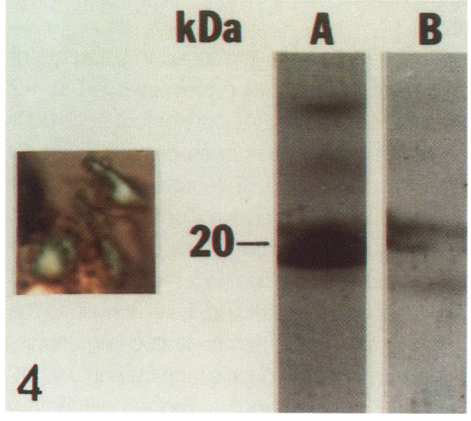


Figure 1. *Experimental production of human AL (λ) amyloid deposits: Renal deposition. (Left) Birefringent Congo-red positive deposits in mouse renal blood vessel wall (polarizing microscopy; ×400). (Middle) Human λ-light-chain deposits in mouse renal blood vessel wall (immunoperoxidase technique; primary antiserum: anti-human λ chain; ×400). (Right) Mouse AP-component deposits in mouse renal blood vessel wall (immunofluorescence technique; primary antiserum: anti-mouse SAP; ×400).*

Figure 3. *Experimental production of renal intratubular non-birefringent Congo-red positive deposits containing human λ light chains and mouse amyloid P component. (Left) Congo red stain (×400). (Middle) Immunoperoxidase staining with anti-human λ-light-chain antiserum (×400). (Right) Immunofluorescence staining with anti-mouse SAP antiserum (×400).*

Figure 4. *Characterization of the human light chains found in the experimentally induced amyloid renal deposits. (Left) The green birefringent Congo-red positive nature of the sediment from the water-soluble extract is shown (×400). (Right) Immunoblot analysis of the human λVI Bence Jones protein injected into mice (lane A) and of the amyloid extract (lane B) (SDS/PAGE followed by immunoblotting with a specific anti-human λVI antiserum; the position of the 20-kDa molecular weight marker is as indicated).*

tiserum, which had been absorbed with pure SAP, or with normal sheep serum, as well as with anti-human SAP antiserum, were negative, i.e., no fluorescence was noted. Furthermore, AP was not present in the kidneys of mice injected with the non-amyloid-associated Bence Jones protein.

Definitive evidence for the amyloid nature of the experimentally induced light-chain deposits was found by electron microscopy. Ultrastructural analysis revealed the presence of 8- to 10-nm nonbranching fibrils comparable in location to the green birefringent deposits depicted in Figure 1. The fibrillar nature of the experimentally induced amyloid was comparable to that found in the renovascular amyloid deposits of a patient with λVI light-chain–associated amyloidosis (Figure 2).

In addition to the presence of vascular and interstitial amyloid in the kidneys of mice injected with the human λ chain, occasional renal tubular casts, located mainly in the loop of Henle, were Congophilic and contained both the injected light chain and mouse AP but did not exhibit green birefringence (Figure 3). Ultrastructural examination of the renal tubular casts did not reveal fibrils.

In an effort to characterize the molecular properties of the human λ-chain protein contained within the experimentally induced amyloid deposits, we extracted this material from the mouse kidney. The protein sediment

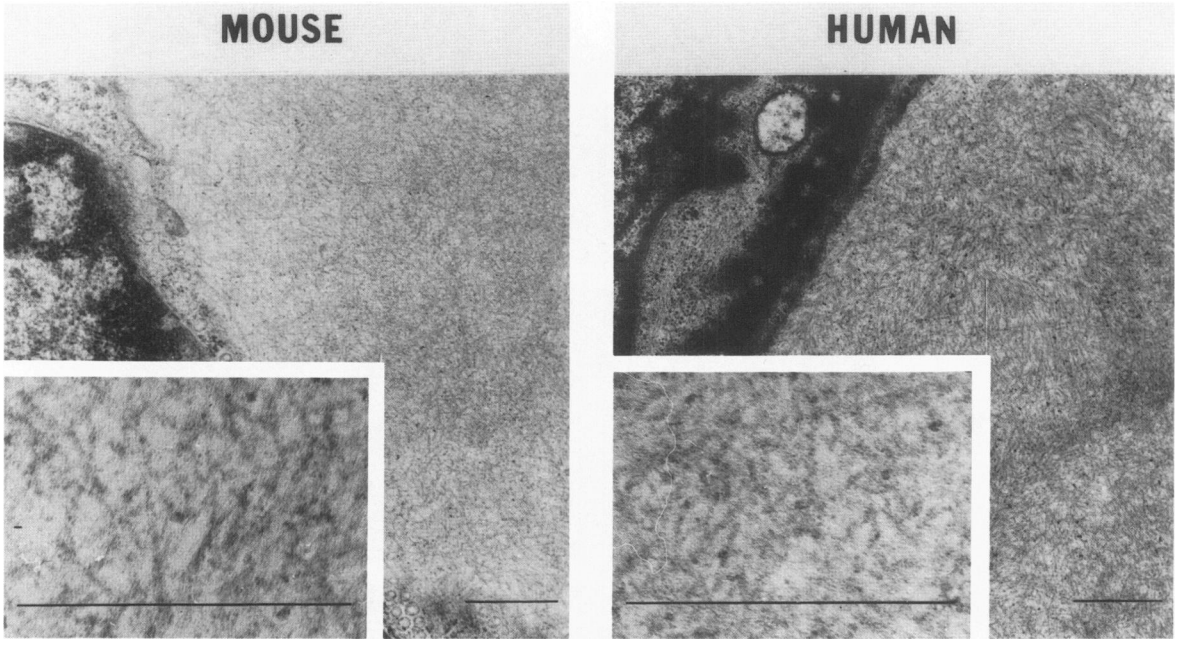


Figure 2. *Fibrillar nature of mouse and human renal blood vessel wall light-chain deposits. Electronphotomicrographs: ×27,500, inset ×84,000; bar = 500 nm.*

obtained after high-speed centrifugation of the water-soluble extract exhibited green birefringence after Congo red staining (Figure 4, left). Examination of this material by SDS/PAGE revealed two protein components: one represented ~80% of the extracted protein and consisted of an ~22-kDa constituent identical in mass to that of the light chain injected; the second was of lower molecular mass (~18- to 20-kDa). Both proteins reacted in immunoblotting experiments with an antiserum specific for human λVI light chains (Figure 4, right), as well as with a general anti-human λ-chain antiserum (not illustrated).

Based on the results obtained with the human amyloid-associated λ light chain, we injected mice with a κ Bence Jones protein obtained from a patient with extensive AL amyloidosis; mice that received 2300 mg of this protein over an 8-week period developed renovascular amyloid deposits that contained the human κ light chain. Of the total dose of protein administered, 700 mg were given over the first 2 weeks in the form of 100-mg injections into nondehydrated mice; the remainder of the protein (1600 mg) was given as 200-mg doses given twice weekly to mice that were dehydrated for 24 hours preinjection. When the amount of protein injected was increased to 4900 mg given over a 12-week period, renal deposition of the human κ chain as amyloid was more extensive and involved not only blood vessel walls but interstitium as well. Furthermore, examination of Congo-red–stained sections of mouse liver, heart, lungs, and spleen revealed perivascular green birefringent deposits (Figure 5). The presence of the human light chain within the Congophilic areas was demonstrated using a specific anti-human κ-chain antiserum (not illustrated). Electron microscopic analysis of the spleen (Figure 6) and kidneys showed the presence of fibrils having comparable ultrastructural features to those found in the mouse injected with the λ Bence Jones protein. Under the same experimental conditions, no renovascular amyloid deposits were found in mice injected over an 8-week period with a non–amyloid-associated Bence Jones protein. After 12 weeks of injection, the only evidence of amyloid formation was the presence of a single renovascular Congophilic deposit exhibiting green birefringence.

Discussion

We have demonstrated that monoclonal light chains obtained from patients with AL amyloidosis, when injected into mice, were deposited as amyloid. The induced deposits had the characteristic tinctorial and ultrastructural features of amyloid, namely, the deposits were Congophilic and exhibited green birefringence by polarizing microscopy and, by electronmicroscopy, were fibrillar in nature. Using specific anti-human κ-or λ-chain antiserum, the deposits were shown to contain human, not mouse, Ig. The vascular and interstitial locations of the amyloid deposits found experimentally were comparable to that found clinically. In contrast to the rapid development

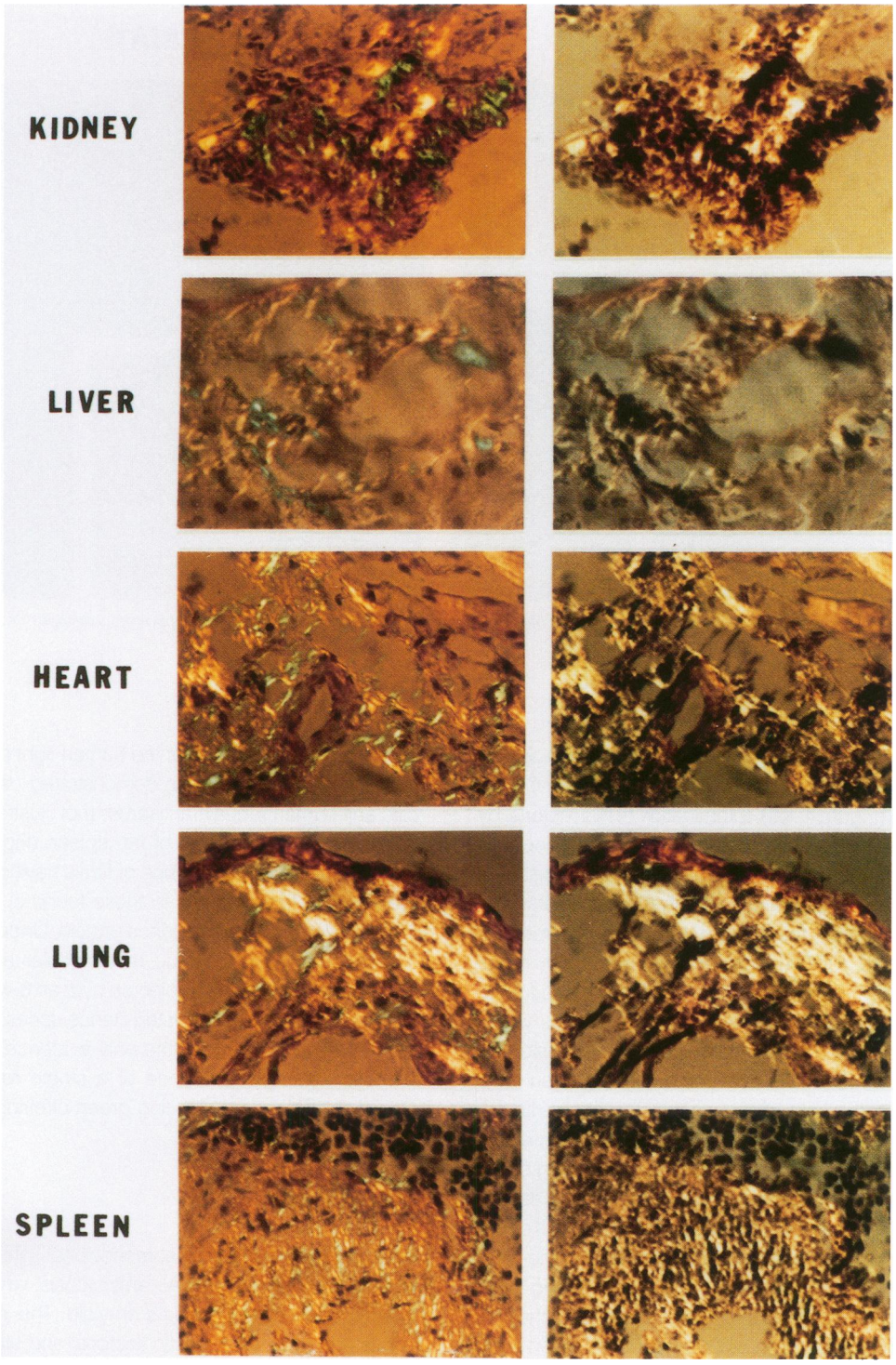


Figure 5. *Experimental production of human AL (κ) amyloid deposits: Systemic deposition: Birefringent Congo-red positive, interstitial deposits in mouse kidney, liver, heart, lungs, and spleen (polarizing microscopy; ×400).*

(within 48 hours) of Bence Jones protein-containing renal tubular casts, crystals, or basement-membrane deposits that resulted from the single injection into mice of 200 to 300 mg of human monoclonal light chains,[12] we found that under the experimental conditions employed, considerably more protein (~2 gm) given over a much longer period (~2 months) was necessary for amyloid formation.

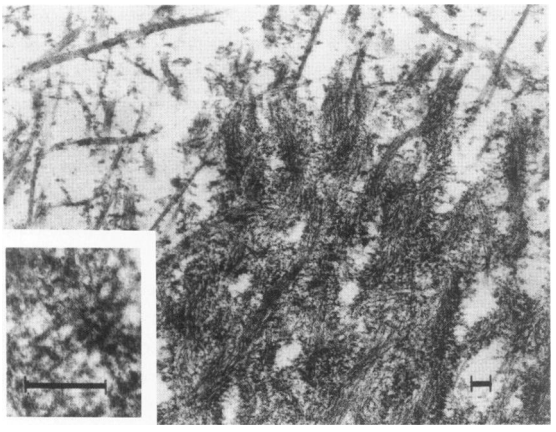


Figure 6. *Fibrillar nature of experimentally induced splenic light-chain deposits. Electronphotomicrographs: ×26,000, inset ×75,000; bar = 100 nm.*

In addition to the human light-chain nature of the experimentally induced amyloid deposits, we demonstrated that these deposits also contained mouse AP. The AP was detected in vascular and interstitial areas that contained birefringent Congophilic fibrillar light-chain deposits; in addition, this component was found in the occasional nonbirefringent Congophilic renal tubular casts that contained the injected human light chain. The homogeneous nature of the deposited light chain may have obscured the detection of fibrils within the proteinaceous casts and, in any case, the sample viewed by electron microscopy was necessarily limited compared with that seen by light microscopy; thus, sparse fibrils may have been missed. Such sparsity could also account for the lack of green birefringence despite Congophilia since it is well recognized that birefringence is dependent on the presence of a sufficient density of amyloid. In this respect, the immunohistochemical identification of AP in amyloid deposits has been shown to be more sensitive than green birefringence for the detection of amyloid.[22]

The contributory role of host AP in amyloid deposition has been evidenced by the demonstration of human AP in porcine insulin-containing amyloid induced at the site of insulin injections in a patient with diabetes mellitus.[23] The presence of mouse AP in the amyloid deposits described here is the first demonstration that mouse SAP binds to human amyloid fibrils and extends to this unique xenogenic model of amyloidosis the invariable association of AP with amyloid deposits. It has long been recognized that a number of precursor proteins can be induced to form amyloid fibril-like structures *in vitro*,[5–6] but whether SAP is required or involved in amyloid fibrillogenesis and deposition *in vivo* remains unresolved, even though there is experimental and clinical evidence that SAP levels and turnover are increased during amyloidogenesis.[24–26]

The finding that under similar experimental conditions Bence Jones proteins obtained from patients with amyloidosis were more readily deposited as amyloid in the mouse (as opposed to a non–amyloid-associated light chain) indicates the inherent amyloidogenicity of certain light chains. A structural basis for light-chain amyloidogenicity has been construed from the prevalence of λ versus κ chains in amyloid deposits, the presence of unusual (hydrophobic) amino acid substitutions in the light-chain variable domain (V_L), and the electronegative nature of amyloid versus non–amyloid-associated protein.[5,27–28] Even more striking is that λ light-chain members of a rare V-region subgroup — $V_{\lambda VI}$ — are preferentially associated with the amyloid process.[18–20] Although the V region of λVI chains contains a subgroup distinctive, two-residue insertion in the third framework region, unusual insertions, deletions, or substitutions are not readily apparent among amyloid-associated λ chains of other V_λ subgroups, as well as among amyloid-associated κ chains. To discriminate amyloid versus non-amyloid light chains on the basis of primary structure, i.e., to identify amyloid-specific residues, is confounded by the inherent V-region amino-acid sequence variability that results from the large number of human V_L genes, recombination of the two V_L-encoding genes, *V* and *J*, and especially, somatic mutation.[29] Whether amyloid-associated light chains have common tertiary structural features that permit the formation of the characteristic β-pleated structure[6] remains to be determined. Conceivably, most or all monoclonal light chains are potentially amyloidogenic, as evidenced by the generation of Congophilic, fibrillar V_L fragments[30–33] and V_L-related peptides[34–36] through enzymatic digestion of Bence Jones proteins. However, the lack of correlation between the *in vitro* production of amyloid and the amyloidogenicity of the same protein *in vivo*[33] suggests that additional (host) factors are required for amyloid formation. Our experimental findings indicate that one factor of pathophysiologic importance — dehydration — which potentiates myeloma cast nephropathy,[21] may also promote light-chain amyloid formation.

In most instances, the major portion of the light-chain protein extracted from the amyloid deposits found in patients with AL amyloidosis consists only of the V_L domain or the V_L plus up to approximately one-half of the light-chain constant domain (C_L); only rarely is the intact protein present.[5–6] Whether these fragments represent synthetic[37] or catabolic products or if a partially degraded light chain is necessary for amyloid formation has not yet been conclusively established. Our analyses of the protein extracted from the experimentally induced λVI-associated amyloid demonstrated that the deposited protein consisted predominately of intact light chains and, to a lesser extent, of a light-chain–related fragment.

Thus, it is conceivable that, due to the inherent susceptibility of the C_L to proteolysis under physiologic conditions,[38] the fragmented light-chain constituents recovered in extracts of light-chain–associated amyloid are the result of tissue proteolysis that occurred after the amyloid was formed.

The ability to reproduce in an animal model the biological and chemical features of human light-chain–associated amyloidosis provides a novel means to determine the amyloidogenic potential of human monoclonal light chains. Furthermore, the availability of an *in vivo* model to investigate the pathogenesis of AL amyloidosis and factors that accelerate (e.g., amyloid enhancing factor[39–41]), prevent, or reverse its formation is of obvious clinical importance.

Acknowledgments

The authors thank Ms. Teresa Williams, Ms. Bobbie Rush, Ms. Mildred Conley, Dr. David Gerard, Ms. Glenys Tennent, and Ms. Melanie Simpson for their assistance with the experimental aspects of this study, and Ms. Julie Ottinger for manuscript preparation.

References

1. Osserman EF, Takatsuki K, Talal N: Multiple myeloma. The pathogenesis of "amyloidosis." Studies on the role of abnormal gamma globulins and gamma globulin fragments of the Bence Jones (L-polypeptide) type in the pathogenesis of "primary" and "secondary amyloidosis," and the "amyloidosis" associated with plasma cell myeloma. Semin Hematol 1964, 1:3–86
2. Isobe T, Osserman EF: Pathologic conditions associated with plasma cell dyscrasias: A study of 806 cases. Ann NY Acad Sci 1971, 190:507–518
3. Isobe T, Osserman EF: Patterns of amyloidosis and their association with plasma-cell dyscrasia, monoclonal immunoglobulins and Bence-Jones proteins. N Engl J Med 1974, 290:473–477
4. Glenner GG, Terry W, Harada M, Isersky C, Page D: Amyloid fibril proteins: Proof of homology with immunoglobulin light chains by sequence analyses. Science 1971, 172:1150–1151
5. Stone MJ: Amyloidosis: A final common pathway for protein deposition in tissues. Blood 1990, 75:531–545
6. Glenner GG: Amyloid deposits and amyloidosis: The β-fibrilloses. N Engl J Med 1980, 302:1283–1292
7. Pepys MB: Amyloidosis. Immunological Diseases, 4th Ed., Vol. 1. Edited by M Samter, DW Talmage, MM Frank, KF Austen, HN Claman. Boston, Little Brown and Co., 1988, pp 631–674
8. Baltz ML, Caspi D, Evans DJ, Rowe IF, Hind CRK, Pepys MB: Circulating serum amyloid P component is the precursor of amyloid P component in tissue amyloid deposits. Clin Exp Immunol 1986, 66:691–700
9. Hawkins PN, Lavender PJ, Pepys MB: Evaluation of systemic amyloidosis by scintigraphy with ^{123}I-labeled serum amyloid P component. N Engl J Med 1990, 323:508–513
10. Zschiesche W, Jakob W: Pathology of animal amyloidoses. Pharmacol Ther 1989, 41:49–83
11. Koss MN, Pirani CL, Osserman EF: Experimental Bence Jones cast nephropathy. Lab Invest 1976, 4:579–591
12. Solomon A, Weiss DT, Kattine AA: Nephrotoxic potential of Bence Jones proteins. N Engl J Med 1991, 324:1845–1851
13. Solomon A, Weiss DT: Experimental production of human amyloidosis AL. Amyloid and Amyloidosis. Edited by Natvig JB, Førre Ø, Husby G, Husebekk A, Skogen B, Sletten K, Westermark P. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1991, pp 193–196
14. Solomon A: Light chains of human immunoglobulins. Meth Enzymol 1985, 116:101–121
15. Solomon A, Weiss DT, Macy SD, Antonucci RA: Immunocytochemical detection of kappa and lambda light chain V region subgroups in human B-cell malignancies. Am J Pathol 1990, 137:855–862
16. Pepys MB: Isolation of serum amyloid P component (protein SAP) in the mouse. Immunology 1979, 37:637–641
17. Pras M, Shubert M, Zucker-Franklin D, Rimon A, Franklin EC: The characterization of soluble amyloid prepared in water. J Clin Invest 1968, 47:924–933
18. Solomon A, Frangione B, Franklin EC: Bence Jones proteins and light chains of immunoglobuins. Preferential association of the $V_{\lambda\ VI}$ subgroup of human light chains with amyloidosis AL(λ). J Clin Invest 1982, 70:453–460
19. Solomon A, Kyle RA, Frangione B: Light chain variable region subgroups of monoclonal immunoglobulins in amyloidosis AL. Amyloidosis. Edited by Glenner GG, Osserman EF, Benditt, EP, Calkins E, Cohen AS, Zucker-Franklin D. New York, Plenum Press, 1986, 449–462
20. Solomon A, Weiss DT: Serologically defined V region subgroups of human λ light chains. J Immunol 1987, 139:824–830
21. Solomon A, Weiss DT: A perspective of plasma cell dyscrasias: clinical implications of monoclonal light chains in renal disease. The Kidney in Plasma Cell Dyscrasias. Edited by Minetti L, D'Amico G, Ponticelli C. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1988, pp 3–18
22. Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB: Specific localization and imaging of amyloid deposits *in vivo* using ^{123}I-labeled serum amyloid P component. J Exp Med 1988, 167:903–913
23. Dische FE, Wernstedt C, Westermark GT, Westermark P, Pepys MB, Rennie JA, Gilbey SG, Watkins PJ: Insulin as an amyloid-fibril protein at sites of repeated insulin injections in a diabetic patient. Diabetologia 1988, 31:158–161
24. Baltz ML, Gomer K, Davies AJS, Evans DJ, Klaus GGB, Pepys MB: Differences in the acute phase responses of serum amyloid P component (SAP) and C3 to injections of casein or bovine serum albumin in amyloid-susceptible and resistant mouse stains. Clin Exp Immunol 1980, 39:355–360
25. Snel FWJJ, Niewold ThA, Baltz ML, Hol PR, van Ederen AM, Pepys MB, Gruys E: Experimental amyloidosis in the ham-

ster: correlation between hamster female protein levels and amyloid deposition. Clin Exp Immunol 1989, 76:296–300

26. Hawkins PN, Wootton R, Pepys MB: Metabolic studies of radioiodinated serum amyloid P component in normal subjects and patients with systemic amyloidosis. J Clin Invest 1990, 86:1862–1869
27. Bellotti V, Merlini G, Bucciarelli E, Perfetti V, Quaglini S, Ascari E: Relevance of class, molecular weight and isoelectric point in predicting human light chain amyloidogenicity. Br J Haematol 1990, 74:65–69
28. Benson MD, Dwulet FE, Madura D, Wheeler G: Amyloidosis related to a λIV immunoglobulin light chain protein. Scand J Immunol 1989, 29:175–179
29. Solomon A: Light chains of immunoglobulins: Structural-genetic correlates. Blood 1986, 68:603–610
30. Glenner GG, Eanes ED, Bladen HA, Terry W, Page DL: Creation of "amyloid" fibrils from Bence Jones proteins *in vitro*. Science 1971, 174:712–714
31. Shirahama T, Benson MD, Cohen AS, Tanaka A: Fibrillar assemblage of variable segments of immunoglobulin light chains: An electron microscopic study. J Immunol 1973, 110:21–30
32. Epstein WV, Tan M, Wood IS: Formation of "amyloid" fibrils in vitro by action of human kidney lysosomal enzymes on Bence Jones proteins. J Lab Clin Med 1974, 84:107–110
33. Linke RP, Zucker-Franklin D, Franklin EC: Morphologic, chemical, and immunologic studies of amyloid-like fibrils formed from Bence proteins by proteolysis. J Immunol 1973, 111:10–23
34. Eulitz M, Linke R: Amyloid fibrils derived from V-region together with C-region fragments from a λII-immunoglobulin light chain (HAR). Biol Chem Hoppe Seyler 1985, 366:907–915
35. Eulitz M, Breuer M, Linke RP: Is the formation of AL-type amyloid promoted by structural peculiarities of immunoglobulin L-chains? Primary structure of an amyloidogenic λ-L-chain (BJP-ZIM). Biol Chem Hoppe Seyler 1987, 368:863–870
36. Eulitz M, Breuer M, Eblen A, Weiss DT, Solomon A: Production of amyloidogenic peptides from human immunoglobulin light (L)-chains. Amyloid and Amyloidosis 1990. Edited by Natvig JB, Førre Ø, Husby G, Husebekk A, Skogen B, Sletten K, Westermark P. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1991, 505–510
37. Buxbaum J: Aberrant immunoglobulin synthesis in light chain amyloidosis. Free light chain and light chain fragment production by human bone marrow cells in short-term tissue culture. J Clin Invest 1986, 78:798–806
38. Solomon A, McLaughlin CL: Bence-Jones proteins and light chains of immunoglobulins. I. Formation and characterization of amino-terminal (variant) and carboxyl-terminal (constant) halves. J Biol Chem 1969, 244:3393–3404
39. Axelrad MA, Kisilevsky R, Willmer J, Chen SJ, Skinner M: Further characterization of amyloid-enhancing factor. Lab Invest 1982, 47:139–146
40. Kisilevsky R, Boudreau BA. Kinetics of amyloid deposition. I. The effects of amyloid-enhancing factor and splenectomy. Lab Invest 1982, 48:53–59
41. Baltz M, Caspi D, Hind CRK, Feinstein A, Pepys MB: Isolation and characterization of amyloid enhancing factor (AES). Amyloidosis. Edited by Glenner GG, Osserman EF, Benditt EP, Calkins E, Cohen AS, Zucker-Franklin D. New York, Plenum Press, 1986, pp 123–128