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Generalized AA-Amyloidosis in a Bat (*Pipistrellus pipistrellus*)

A. D. Gruber and R. P. Linke

Abstract. Generalized amyloidosis was found to be the cause of death in a female adult insectivorous pipistrelle bat (*Pipistrellus pipistrellus*) after chronic wound inflammation. Large amounts of amyloid were detected in liver, spleen, kidneys, stomach, intestine, lymphatic tissues, and endocrine and salivary glands. Congo red staining and green birefringence identified amyloid; the Congo red staining was sensitive to potassium permanganate oxidation. The amyloid was further classified immunohistochemically. The deposits reacted with two anti-human-AA-amyloid monoclonal antibodies in a peroxidase–antiperoxidase reaction, whereas no reaction was found with antibodies specific for other types of amyloid. Thus, the bat amyloid deposits were identified as generalized reactive AA-amyloidosis.

Key words: AA-amyloidosis; anti-amyloid antibodies; bat; generalized amyloidosis; immunohistochemistry.

Amyloidosis has been extensively studied in domestic and laboratory mammals[3,13] and captive birds.[14] In these species, comprehensive information is available concerning species susceptibility, type of amyloid, tissue distribution patterns, and pathogenetic factors with distinct species variations. Only few cases of systemic amyloidosis have been reported in wild animals, for example, raccoons,[1] badgers,[2] hares,[2,5] martens,[4] and a gazelle.[8] In most cases, these were casual findings with uncertain clinical history and the pathogenesis remained questionable. Identification of the type of amyloid was possible in some cases by taking advantage of immunohistochemical cross-reactivity of antibodies directed against defined types of human amyloid.[7–9,11,14] Until now, there were no reports on amyloidosis in bats. The present report describes reactive generalized amyloidosis in a pipistrelle bat with clinical history of chronic wound inflammation and immunohistochemical classification as AA-amyloidosis.

An adult female insectivorous pipistrelle bat (*Pipistrellus pipistrellus*; ulna length 3.2 cm) was accidentally injured causing amputation of the left forearm. The bat was surgically treated. After initial good recovery, the animal developed progressive anorexia 6 months following the surgery and died.

Gross pathologic evaluation revealed an emaciated carcass with body weight of 4.0 g and few ectoparasites (mites). Most parts of the left forearm were missing. The humerus was reduced to a stub of approximately 1 cm that barely stood out of the mildly swollen surrounding tissue. The liver and spleen were severely enlarged and inhomogenously pale.

Histologic examination was performed after formalin fixation, paraffin embedding, and hematoxylin and eosin staining of dewaxed and rehydrated tissue sections. The skin, connective tissue, and musculature around the humeral stub had severe chronic suppurative to granulomatous inflammation. A migrating nematode (approximately 100 μm in diameter) and few sporulated eggs (approximately 11 × 24 μm) were noted in this granulation tissue, the species of which could not be identified. In the liver, large amounts of a hyaline, eosinophilic material were deposited around central veins and vessels in the portal triads protruding into the spaces of Disse in the perilobular areas (Fig. 1a). The kidneys had heavy global glomerular hyaline depositions and broadening of the tubular interstitium. Severe hyaline eosinophilic accumulations were found in the spleen, predominantly in large cuffs around depleted periarteriolar lymphoid sheaths and in the walls of small arterioles. The adrenal cortex, thyroid, and salivary and supraorbital glands had heavy infiltrations of the interstitium and small vessel walls. Strong interstitial deposits were seen in the lamina propria of the digestive tract, mainly in the glandular stomach and small intestine (Fig. 2a). Varying numbers of small vessel walls were affected in the leptomeninges, heart and skeletal muscles, lymphatic tissues, pancreas, and lung. All protein deposits stained with Congo red and were green birefringent in polarized light.[10] Both congophilia and green birefringence were sensitive to potassium permanganate oxidation.[12]

The amyloid deposits of the bat were classified immunohistochemically with a panel of antisera and monoclonal antibodies directed against different types of human amyloid using the peroxidase–antiperoxidase method [6,9,11] (Table 1). The reactivity was visualized with 3-amino-9-ethyl-carbazole as chromogen. Tissue sections were counterstained with Mayer's acid hemalum. Very strong immunohistochemical reactions were obtained with anti-AA monoclonal antibody mc4 in the liver (Fig. 1b), spleen, kidneys, digestive tract (Fig. 2b), lymphatic tissues, various exocrine and endocrine glands, and small vessels in numerous tissues, whereas anti-AA monoclonal antibody mc13 stained somewhat weaker than mc4. The tissue distribution pattern of both antibodies (mc4, mc13) was virtually identical. No reactions were demonstrated with other antibodies or antisera (Table 1) and positive reactions on respective human antigen controls proved the specificity of the assay.

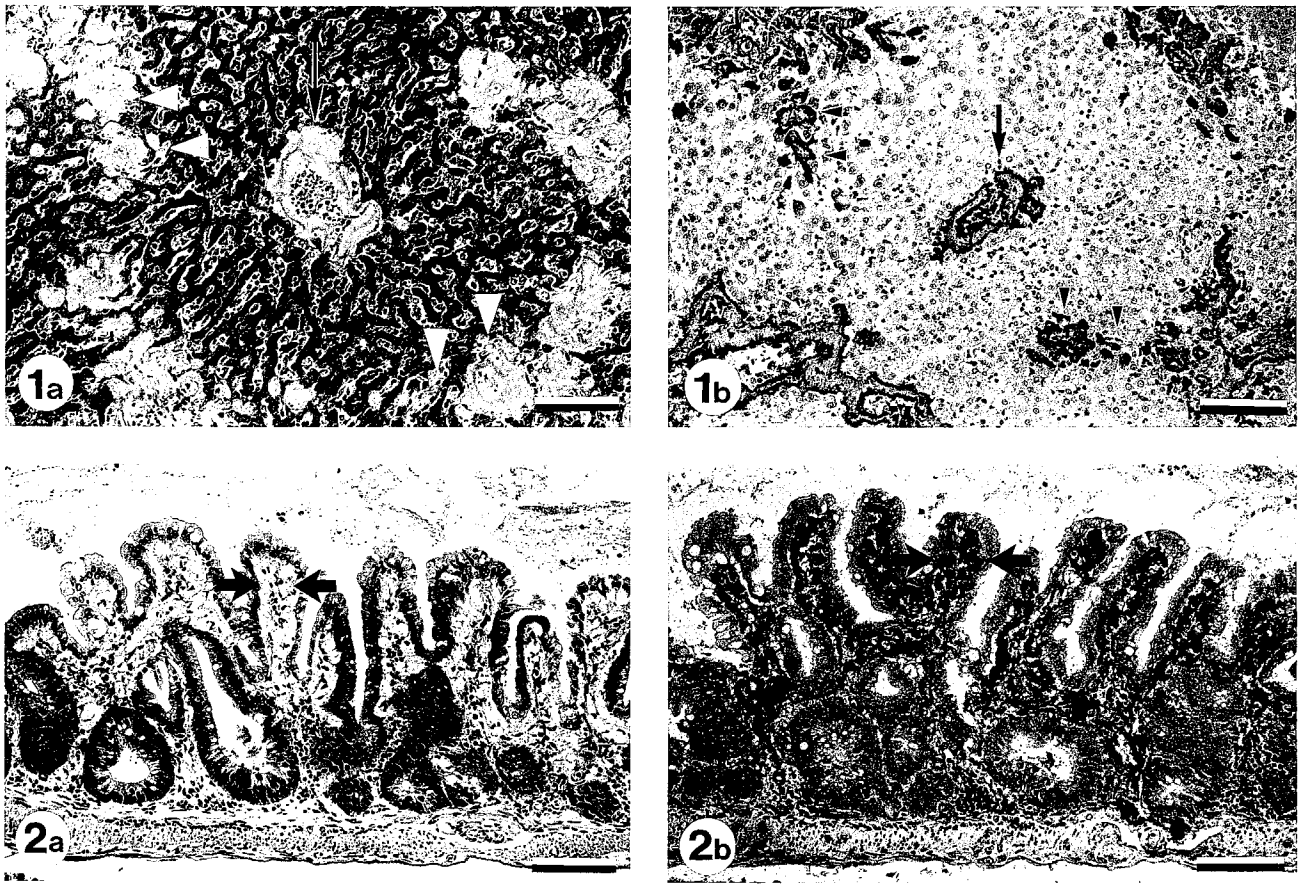

Fig. 1. Liver; pipistrelle bat. Severe amyloid deposits around the central vein (arrow) and around vessels in the Glisson's triad protruding into the spaces of Disse in the perilobular areas (arrowheads). (a) HE and (b) peroxidase–antiperoxidase method using anti-human-AA-amyloid monoclonal antibody mc4 with amino-ethyl-carbazole as substrate and Mayer's acid hemalum counterstain. Bar = 100 μm.

Fig. 2. Jejunum; pipistrelle bat. Severe amyloid deposits in the mucosal lamina propria (arrows). (a) HE and (b) peroxidase–antiperoxidase method using anti-human-AA-amyloid monoclonal antibody mc4 with amino-ethyl-carbazole as substrate and Mayer's acid hemalum counterstain. Bar = 100 μm.

Based on the histomorphological and immunohistochemical results, the depositions were shown to represent a generalized AA-amyloidosis with multiple organ manifestation. In this bat, the cause of the abundant deposition of serum amyloid A derivates may be associated with the chronic wound inflammation following accidental amputation of the left forearm (reactive AA-amyloidosis).[3] AA-amyloidosis reported in other domestic and wild animal species including birds frequently occurs without recognizable underlying inflammation (idiopathic AA-amyloidosis) with distinct species variations.[3,4,8,13,14] The tissue distribution pattern of amyloid in this bat is consistent with the typical distribution of reactive AA-amyloidosis in man and other species.[3–5,8,13,14] Emaciation and death in this bat were most likely caused by renal failure with uremia due to extensive renal amyloid deposition. Additionally, insufficiency of other organs (e.g., of the intestine and liver) may have contributed to the death. The pathogenetic role of the parasitic infestation of the chronic granulation tissue remains unclear. A putative causal association of reactive AA-amyloidosis and parasitic infections was discussed previously[3–5,13] but needs further investigations.

Reactivity with two anti-human-AA-amyloid monoclonal antibodies proved the identity of the deposited pipistrellus protein as AA-amyloid. Cross-reaction of anti-human-AA-amyloid antibodies with AA-amyloid in animals has been proven to be a suitable diagnostic tool in numerous species.[5,8,11,14] Antigenic relationship among different mammals was also shown even in AL-amyloid using a polyclonal antiserum.[7,9] In this study, only two out of five monoclonal antibodies against human AA-amyloid exhibited positive immunohistochemical reactivity, suggesting differences in the evolutionary conservation of different epitopes of the AA-amyloid fibril.

In order to find out whether amyloidosis in the bat has been reported before, all entries on amyloidosis documented since approximately 1970 by the following databases were screened: Biological Abstracts, Embase, Medline, SciSearch, and those 71 databases represented by DIMDI. Among 56,570 entries on amyloidosis, no entry relating to bat amyloidosis

Table 1. Reactivity of a panel of monoclonal antibodies and antisera directed against different human amyloid types with amyloid in numerous tissues (see text) of a pipistrelle bat.

Antibodies[6,9]	Against Amyloid Class*	Antibody Dilution†	Animal Immunized	Immunohistochemical Reaction‡
Anti-AA mcl§	AA	1:10	mouse	−
Anti-AA mc4§	AA	1:10	mouse	+++
Anti-AA mcl3§	AA	1:10	mouse	++
Anti-AA mc29§	AA	1:10	mouse	−
Anti-AA mc31§	AA	1:10	mouse	−
Anti-Aλ‖ (HAR)#	Aλ	1:8,000	rabbit	−
Anti-Aκ‖ (SIN)#	Aκ	1:4,000	rabbit	−
Anti-AF‖ (TIE)#	ATTR	1:2,000	rabbit	−
Anti-AB‖ (WOE)#	$A\beta_2m$	1:800	rabbit	−

* Abbreviations: AA, amyloid A; Aλ, amyloid fibril proteins of immunoglobulin lambda light chain origin; Aκ, amyloid fibril proteins of immunoglobulin kappa light chain origin; ATTR, amyloid fibril proteins of transthyretin origin; $A\beta_2m$, amyloid fibril proteins of beta$_2$-microglobulin origin.

† Primary antibody (cell culture supernatant; serum).

‡ Intensity of reaction: − no, ++ strong, +++ very strong.

§ Monoclonal antibody.

‖ Polyclonal antiserum.

Patient's name abbreviated.

could be found. According to this search and the reviews on animal amyloidosis[3,13] we regard our contribution as the initial report on amyloidosis in a bat.

The data indicate that severe generalized reactive AA-amyloidosis occurs in bats and that cross-reacting monoclonal antibodies and potassium permanganate oxidation prior to Congo red staining are suitable tools for identification of AA-amyloidosis in the bat.

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