

# SPECT Imaging of Systemic Amyloidosis with <sup>125</sup>I-scFv to Heparan Sulfate

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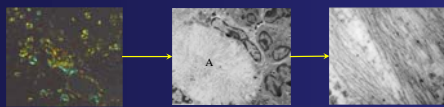
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## Introduction

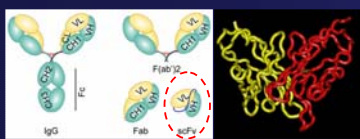
Amyloid diseases result from tissue deposition of fibrillar aggregates. Amyloid is associated with a broad spectrum of disorders including monoclonal gammopathies, chronic inflammation, type 2 diabetes, and Alzheimer's disease. In all cases the formation of amyloid fibrils is the underlying etiology or is a complicating factor in the course of the disease. In addition to protein fibrils, amyloid deposits contain a plethora of associated molecules, including high concentrations of heparan sulfate proteoglycans.

Currently, efforts to image amyloid utilize biomarkers that target the protein fibril component. As an alternative approach we have evaluated radioiodinated single chain variable fragments (scFv) that bind heparan sulfate for their ability to image amyloid deposits *in vivo*. scFv's were isolated by panning the Nissim phage display library on heparan sulfate extracted from bovine kidney or human skeletal muscle glycosaminoglycans.

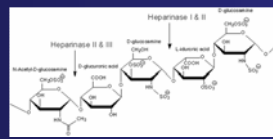
Following radioiodination <sup>125</sup>I-scFv's were administered to animals with severe systemic inflammation-associated AA amyloidosis or to amyloid-free mice. In disease-free mice certain of the <sup>125</sup>I-scFv tested bound specifically, in mice without amyloid, to heparan sulfate naturally occurring in the renal tubules as well as glomerular tufts and capsules. In addition, catabolism and dehalogenation of the scFv in the kidney liberated free <sup>125</sup>I iodide that accumulated in the stomach at 4h post-injection as evidenced in microSPECT images. In contrast, <sup>125</sup>I-scFv injected into AA mice deposited the radioisotope in the liver, kidney, heart, pancreas, spleen, and intestine. Microautoradiography further revealed that in the presence of amyloid the scFv preferentially associated with the pathologic deposits in all organs. These data indicate that even though heparan sulfate is expressed in normal tissue, scFv for specific heparan sulfate structures are suitable for imaging amyloid disease at 4h post injection.



AA amyloidosis is associated with chronic inflammation of bacterial or non-pathogenic etiology. In response to pro-inflammatory stimuli such as lipopolysaccharide or interleukin-6 (IL-6), acute phase proteins, including serum amyloid protein A (sAA), the precursor of AA amyloid fibrils, are synthesized and secreted by the liver. In mice, amyloid deposits occur in the spleen, liver, pancreas, heart, kidneys, and vasculature. Amyloid appears microscopically as green birefringent material when stained with Congo red and as a collection of unbranching fibrils ~10 nm in diameter, in the electron microscope.

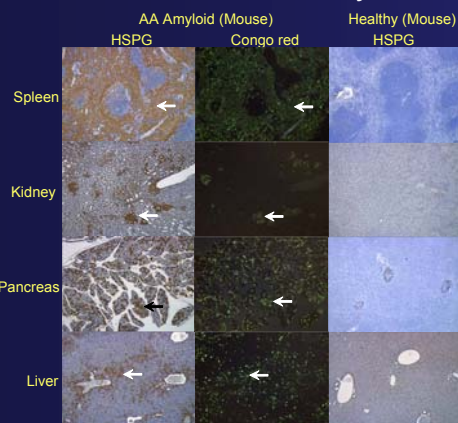


Schematic (left) and x-ray crystallographic (right) structure of single chain immunoglobulin variable fragment (scFv). These ~28 kDa molecules contain a specific monovalent binding site and are cleared rapidly from the circulation via the kidneys.



Heparan sulfate (HS) is a polysaccharide, generally less sulfated than monomeric heparin, that is ubiquitously found in all mammalian tissues as proteoglycans, e.g., perlecan. HS is invariably associated with all amyloid deposits.

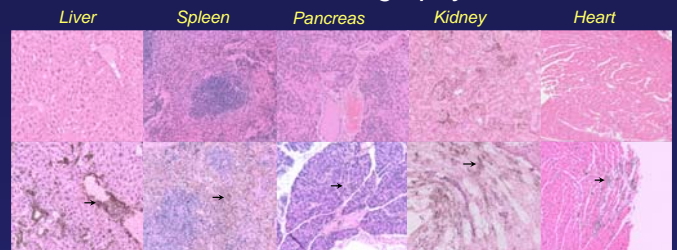
## Heparan sulfate proteoglycan (HSPG) is highly concentrated in AA amyloid deposits in mice



Co-localization of HSPG with tissue AA amyloid in mice. HSPG immunostained with specific mAb (rat IgG anti-human HSPG) was evidenced in the tissues by the brown diaminobenzidine (arrows indicate certain specific regions of co-localization). Amyloid deposits were visualized directly by the green birefringence in the Congo red-stained section. NOTE: In healthy, amyloid-free mice HSPG is barely visible in these tissues at this low magnification and dilution of the mAb. Original mag x100.

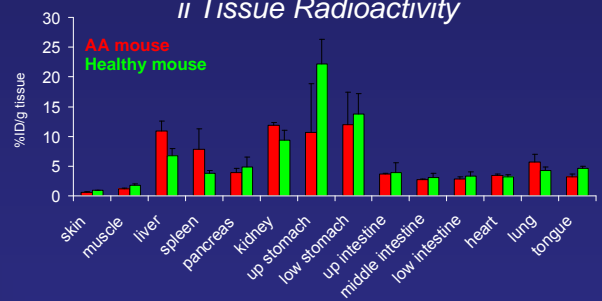
## HS-reactive scFv binds AA amyloid in the liver, spleen, kidneys, pancreas, and heart

### i Autoradiography



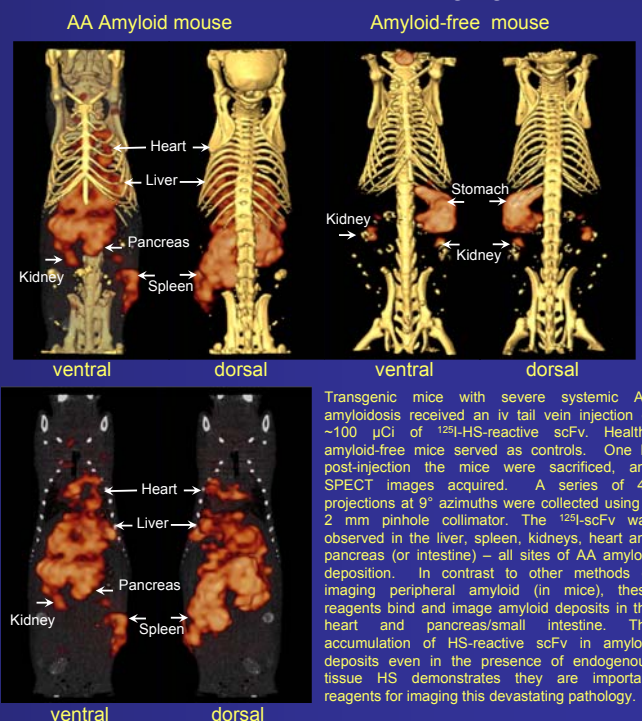
Co-localization of <sup>125</sup>I-labeled HS-reactive scFv with AA amyloid deposits occurred in all tissues examined by autoradiography. In healthy amyloid-free mice (upper panel) no scFv binding was observed with the exception of renal tubules and the glomerular capsule. In mice with AA (lower panel), scFv was observed associated with amyloid in all tissues that contained Congoophilic deposits, including the heart and pancreas. No significant binding was observed to cells or tissues devoid of amyloid. Original mag. X 160

### ii Tissue Radioactivity



Localization of <sup>125</sup>I-labeled HS-reactive scFv was quantified by measuring the activity associated with organs and tissues collected post-mortem and expressed as % injected dose per gram tissue. The liver and spleen have considerably more <sup>125</sup>I-scFv in the mouse with amyloid as compared to the control. In contrast the stomach of the healthy mouse has more activity, indicative of the accumulation of liberated iodide.

### iii SPECT/CT Micro Imaging



Transgenic mice with severe systemic AA amyloidosis received an iv tail vein injection of ~100 µCi of <sup>125</sup>I-HS-reactive scFv. Healthy amyloid-free mice served as controls. One hr post-injection the mice were sacrificed, and SPECT images acquired. A series of 40 projections at 9° azimuths were collected using a 2 mm pinhole collimator. The <sup>125</sup>I-scFv was observed in the liver, spleen, kidneys, heart and pancreas (or intestine) – all sites of AA amyloid deposition. In contrast to other methods of imaging peripheral amyloid (in mice), these reagents bind and image amyloid deposits in the heart and pancreas/small intestine. The accumulation of HS-reactive scFv in amyloid deposits even in the presence of endogenous tissue HS demonstrates they are important reagents for imaging this devastating pathology.