Published in final edited form as:

Methods Enzymol. 2006 ; 412: 161–182.

Micro-Imaging of Amyloid in Mice

Jonathan S. Wall^{1,§}, Michael J. Paulus², Shaun Gleason², Jens Gregor³, Alan Solomon¹, and Stephen J. Kennel⁴

¹Human Immunology & Cancer Program, Department of Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN, USA jwall@mc.utmck.edu; Tel: (865) 544 9165; Fax: (865) 544 6865 asolomon@mc.utmck.edu; Tel: (865) 544 9165; Fax: (865) 544 6865

²Siemens Medical Solutions Molecular Imaging, LLC, 810 Innovation Drive, Knoxville, TN, USA Michael.Paulus@ctimi.com; Tel: (865) 218 1621 Shaun.Gleason@ctimi.com; Tel: (865) 218 1642

³Department of Computer Science, University of Tennessee, 1122 Volunteer Blvd., Suite 203, Knoxville, TN 37996-3450 jgregor@cs.utk.edu; Tel: (865) 974 4399; Fax (865) 974 4404

⁴Oak Ridge National Laboratory, Life Sciences Division, Bldg 4500S, Rm F150, Oak Ridge, TN kennelsj@ornl.gov; Tel: (865) 574 0825; Fax: (865) 576-7651

Abstract

Scintigraphic imaging of radioiodinated serum amyloid P-component is a proven methodology for the clinical detection of peripheral amyloid deposits (Hawkins, *et al.*, 1990). However, the inability to perform comparably high resolution studies in experimental animal models of amyloid disease has impacted not only basic studies into the pathogenesis of amyloidosis but also in the preclinical *in vivo* evaluation of potential anti-amyloid therapeutic agents. We have developed micro-imaging technologies, implemented novel computational methods and established protocols to generate high resolution images of amyloid deposits in mice. ¹²⁵I-labeled serum amyloid P component (SAP) and an amyloid-fibril reactive murine monoclonal antibody (designated 11-1F4) have been employed successfully to acquire high resolution single photon emission computed tomographic (SPECT) images that, when fused with x-ray computed tomographic (CT) data, have provided precise anatomic localization of secondary (AA) and primary (AL) amyloid deposits in mouse models of these diseases.

This chapter will provide detailed protocols for the radio-iodination and purification of amyloidophilic proteins and the generation of mouse models of AA and AL amyloidosis. A brief description of the available hardware and the parameters used to acquire high-resolution microSPECT and CT images is presented, and the tools used to perform image reconstruction and visualization that permit the analysis and presentation of image data are discussed. Finally, we provide established methods for measuring organ and tissue specific-activities with which to corroborate the microSPECT and CT images.

I Introduction

The amyloidoses represent a growing number of diverse, insidious and devastating diseases which comprise cerebral forms such as Alzheimer's disease (Bishop and Robinson, 2004; Cummings, 2004) and spongiform encephalopathies (Ghetti *et al.*, 2003) as well as peripheral disorders including type II diabetes (Hull *et al.*, 2004), primary (AL) (Bellotti *et al.*, 2000; Merlini and Bellotti, 2003) and secondary (AA) amyloidosis (Rocken and Shakespeare, 2002). All are characterized by the aggregation of normally soluble, natural proteins or their

[§]Corresponding Author: Jonathan Wall University of Tennessee Graduate School of Medicine 1924 Alcoa Highway Knoxville, TN 37920 Tel: (865) 544 9165; Fax: (865) 544 6865 jwall@mc.utmck.edu

fragments into highly ordered amyloid fibrils that deposit within vital organs and tissues leading to dysfunction and if unremitting, death.

Monitoring the pathogenesis of these diseases and their response or resistance to treatments and preventative interventions has relied historically upon the histologic evaluation of tissue biopsies, surveys of surrogate physiological markers, such as organ function, and post-mortem histological examination. This is also true in the fields of preclinical and basic research that use experimental animal models of amyloidosis (Kisilevsky et al., 1995; Kisilevsky et al., 2004; Mihara et al., 2004; Stenstad and Husby, 1996; Wall et al., 2001; Zhu et al., 2001). Within the last two decades however, advances in clinical medical practices have made possible, by whole body scintigraphic examination [planar 2-D imaging of a bodily radiation source], the detection of amyloid deposits in patients using radioiodinated serum amyloid P-component (SAP) (Hawkins et al., 1988a; Hawkins et al., 1988c; MacRaidl et al., 2004; Pepys et al., 1997). To date, radioiodinated SAP scintigraphy has been performed in over 1000 patients and is currently the most universally employed clinical modality for determining the scope and biodistribution of amyloid deposits in patients with non-cerebral amyloidosis (Hawkins, 2002).

Due to its high affinity for fibrils and short lifetime in the circulation, human SAP is an excellent agent for imaging amyloid *in vivo*. Indeed, human SAP exhibits a higher affinity for mouse AA-amyloid fibrils *in vivo* than the endogenous murine SAP making it an exceptional preclinical tracer for imaging amyloid in murine models (Hawkins et al., 1988b). We have used human ¹²⁵I-SAP to generate quantitative microSPECT/CT images of hepatic and splenic AA amyloid deposits in a transgenic murine model.

In addition to SAP, we have successfully imaged, in mice, localized human AL amyloidomas using the amyloid-reactive monoclonal antibody (mAb) 11-1F4 (Hrncic et al., 2000; O'Nuallain et al., 2005; Solomon et al., 2003a; Solomon et al., 2003b) as an imaging agent. The ¹²⁵I-labeled 11-1F4 mAb, when injected iv into mice bearing amyloidomas composed of human AL fibrils, was seen in the co-registered SPECT/CT images to localize almost exclusively within the amyloid (Wall et al., 2005b).

II Microimaging Apparatus

A number of microCT and microSPECT small animal imaging machines are commercially available; however, at present there are only 2 manufacturers of dual (combined) microSPECT/CT apparatus. For a list of preclinical micro imaging systems with links to the relevant manufacturers the reader is referred to the molecular imaging site maintained by Stanford University (www.mi-central.org).

For our experiments we have exclusively used microSPECT and CT systems, designed and fabricated by Mike Paulus and Shaun Gleason while at Oak Ridge National Laboratory (Oak Ridge, TN) (Paulus et al., 2001; Paulus et al., 2000a; Paulus et al., 2000b; Paulus et al., 1999). The microCT and recently developed dual-modality microSPECT/CT systems are now commercially available (CTI Concorde Microsystems, Knoxville, TN).

The microCT system contains a 20-80 kVp x-ray source with 50 micron focal spot and a 2048 × 3072 charge coupled device (CCD) array detector, optically coupled to a Gadox (Gd₂O₂S (Tb)) phosphor screen via a fiber-optic taper. The source and detector are mounted on a circular gantry allowing it to rotate 360° around the subject (mouse) positioned on a stationary bed (Fig 1.). A number of detectors are now commercially available; in our studies the detector can capture a 90 mm × 60 mm field of view, more than adequate for a whole-body mouse study. A CT dataset, comprised of 360 projections, can be acquired in approximately 6 minutes.

The microSPECT apparatus used to collect the I-125-labeled SAP and 11-1F4 mAb images was designed specifically for small animal imaging and fabricated at Oak Ridge National Laboratory and situated immediately next to the microCT machine. The SPECT detector consists of an array of multi-anode photomultiplier tubes coupled to a pixilated NaI(Tl) crystal array (Jefferson Laboratory, Newport News, VA) which revolves around the mouse subject in typically 6° increments. The detector has a 10 × 20 cm active area which is discretized into a 50 × 100 array. The resolution is approximately 2.5 mm when coupled with a high resolution parallel-hole collimator. MicroSPECT datasets are comprised of 60 projections that are collected over the course of 30 to 60 minutes – depending upon the specific activity of the tracer and its concentration at the site of the amyloid.

III Protein Tracers

The human SAP used in our studies was kindly provided by Prof. Philip Hawkins (Department of Medicine, Royal Free and University College Medical School, London, UK) and was of clinical grade. The protein was isolated from heat-treated human serum as previously described, by calcium-dependent affinity chromatography (De Beer and Pepys, 1982). The protein was stored frozen until used.

Murine 11-1F4 mAb was generated in our laboratory by inoculating mice with intra-peritoneal injection of 1×10^6 11-1F4-producing hybridoma cells followed by isolation of the antibody from ascitic fluid by size exclusion and ion-exchange chromatography (Abe et al., 1993a; Abe et al., 1993b). Certain murine 11-1F4 preparations were produced by the National Cancer Institute (Biopharmaceutical Development Program, NCI-Frederick) under the Rapid Access to Intervention Development (RAID) program. All 11-1F4 samples were stored frozen until used.

IV Preparation of Radio Iodinated proteins

Although unsuitable for the clinic, ^{125}I has been used in our small animal imaging studies because of its relatively long half-life (60 d), ease of labeling and availability - the long half life also facilitates post mortem autoradiographic analyses of tissue sections. In the clinical arena, the low energy of the ^{125}I gamma ray is unsatisfactory - the attenuation length of a 35 keV gamma ray in soft tissue is about 3.3 cm – small relative to the diameter of a human. For mouse studies, however, the attenuation length of ^{125}I is greater than the largest axial diameter of a typical adult mouse and therefore more than adequate for providing high-resolution images. Indeed, the attenuation of the 35 keV gamma rays in mice is substantially less than that of the gamma rays emitted by standard clinical imaging isotopes (e.g., ^{201}Tl , $^{99\text{m}}\text{Tc}$) in humans.

Our imaging experiments have utilized radio-iodinated proteins. Whether proteins are labeled electrochemically, enzymatically or via chemical oxidation (ICl, iodogen, or chloramine T) the iodide atoms are attached predominately to either ortho or para positions on tyrosine rings. However, iodotyrosines are subject to dehalogenation *in vivo*, particularly within the liver (Auf dem Brinke et al., 1980; Kohrle et al., 1990; Spanka et al., 1990). The loss of iodide from the targeting molecule does not necessarily negate imaging and biodistribution studies particularly for relatively short time frames and for targets in organs other than liver. We have not found fundamental differences in the performance of targeting proteins radio-iodinated by any of the oxidative methods mentioned above. For simplicity we chose to conduct radio-iodination with limiting amounts of Chloramine T, as described below.

In the event that dehalogenation proves problematic several chemical approaches have been designed to alleviate the problem (De Nardo et al., 1988; Hnatowich, 1990; Vaidyanathan et al., 2000; Vaidyanathan and Zalutsky, 1990a; Vaidyanathan and Zalutsky, 1990b). These involve attachment of the iodide to benzoyl rings which are subsequently coupled to proteins

via N-hydroxy-succinimide (NHS) reactions with lysine amino groups. Note that the Bolton Hunter reagent, although it employs NHS coupling is NOT resistant to dehalogenation reactions since the iodide is still conjugated to the ortho and para positions on a tyrosyl moiety (Bolton and Hunter, 1973). As an alternative to the Chloramine T radio-iodination, we describe a procedure for radio-iodination with succinimidyl-4-iodo benzoate (SIB) (Vaidyanathan and Zalutsky, 1990a). This reaction results in stably labeled compounds, but generally yields probes with lower specific activity due to the relatively inefficient coupling via the NHS reaction.

1 Procedure for the Chloramine T radio iodination of proteins

Many methods for radio-iodination have been published previously (Bailey, 1994; Behr et al., 2002; Garg et al., 1993; Karonen, 1990; Vaidyanathan and Zalutsky, 1990a; Vaidyanathan and Zalutsky, 1990b; Wilbur, 1992). We prefer a modification of the method of McConahey and Dixon (McConahey and Dixon, 1966). Proteins of any size can be radio-iodinated by these methods if they contain an available tyrosine. Purification of the final product free of aggregates and free iodide is normally achieved by size exclusion gel filtration on media suitable for separating the radiolabeled protein from contaminants. A stepwise procedure follows:

1. In a monitored radioactive work zone, assemble microfuge tube, micropipettes, buffers, protein, radioiodide and chemicals - see below. Always use appropriate protective clothing, gloves and eyewear.
2. Transfer 5 μ L of 0.5 M sodium phosphate pH 7.6 to a clean microfuge tube (1.5 mL capacity).
3. Pipette radioactive iodide (Perkin Elmer protein iodination grade 125 I) into the buffer. Up to 50 μ L of radioiodide can be used in this reaction. If more of the iodide solution is required, increase the amount of sodium phosphate buffer accordingly.
4. Add protein as a concentrated solution freshly dialyzed into 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.6 (PBS). Proteins can also be radiolabeled in Tris-HCl buffered saline or other non reducing buffered solutions.
5. Add 5 μ L of 1 mg/mL freshly prepared Chloramine T (Sigma, St Louis, MO) - dissolve 1 or 2 mg of dry crystals in the appropriate volume of water immediately prior to addition to the labeling mix. For reactions with more than 0.5 mg of protein an additional 5 μ L of chloramine T solution should be added for each additional 0.5 mg protein.
6. The reaction is complete within 1 minute; however, residual reaction should be quenched by addition of 5 μ L of 2 mg/mL $\text{Na}_2\text{S}_2\text{O}_5$ [sodium metabisulfite, (J.T.Baker, Phillipsburg, NJ)] - freshly dissolved in water immediately before use.
7. For size exclusion gel filtration, a dedicated HPLC can be used; however, low yields of protein may result. For small amounts of protein we recommend an open, disposable column method with media eluting under gravity. Pour a column with a 5 mL bed volume in a disposable plastic 5 mL pipette plugged with glass wool. For whole IgGs or SAP, use Ultrigel AcA 34 resin (Sigma, St Louis, MO) equilibrated with PBS containing 5.0 mg/mL BSA or 1.0 mg/mL gelatin as carrier protein. For smaller proteins such as scFv Ultrigel AcA 44 can be used.
8. Add 50 μ L of PBS with 5 mg/mL BSA and 20 μ L of 10 mg/mL Blue Dextran (Pharmacia Fine Chemicals) to the quenched radio-iodination reaction mixture and load onto the gel filtration column.
9. Manually collect 1 min fractions (approximately 200 μ L) in plastic tubes. Count a small volume of each fraction ($\sim$ 1-2 μ L) in a gamma scintillation counter using

the ^{125}I program. Pool 3 or 4 fractions with the highest activity and test for activity and radiochemical purity.

10. For immunoreactivity of radio-iodinated antibodies, specific antigen tests should be done. Such a test for reactivity of 11-1F4 antibody is described below.
11. Radiochemical purity of proteins is checked on SDS-PAGE gels. Small fractions of the pooled material (often as little as 0.1 μL) are separated in reducing conditions on 10% acrylamide SDS-PAGE. The gels are not fixed or washed, but are covered with plastic wrap and placed on phosphorimager screens (e.g. Cyclone Storage Phosphor System, Perkin Elmer, Shelton, CT). The resultant images are analyzed by region of interest quantitation. The preparations should be >95% pure.

2 Comments

Highly concentrated reactions are more efficient. Addition of large volumes of iodide solution or of proteins not adequately dialyzed can introduce agents (presumably reductants) which inhibit the reaction. Optimization of the methods described above can provide yields of up to 50% radioiodide incorporation in the final product. Using these techniques we have successfully radio-iodinated protein amounts from 0.1 μg to >10 mg.

3 Immunoreactivity Protocol for radiolabeled 11-1F4 mAb

This assay describes a bead-conjugated antigen binding assay which has been used to confirm the immunoreactivity of the 11-1F4 mAb. This is a large format assay to measure the binding of radiolabeled 11-1F4 to antigen-coated beads and is appropriate for various radio-iodinated forms. It is recommended that duplicate or triplicate samples are tested at two antibody concentrations.

1. Bead preparation: Polystyrene beads (0.91 μm -diameter, conjugated with amino groups) are obtained from Spherotech Inc (Libertyville, IL). Approximately 0.5 mL of bead solution is washed three times in PBS by centrifugation for 2 min at $10,000 \times g$ and resuspended by pipetting using a 200 μL micropipette with a small bore tip. The washed beads are then treated with 4.0 mL of 0.5% glutaraldehyde (freshly diluted from a 25% stock solution) in PBS for 5 min at RT and then washed 3 times as above. After the final wash the beads are resuspended in 4 mL PBS containing 4 mg of peptide antigen (approximately 1.2 μmoles) and tumbled end-over-end for 18 hrs at RT. To block free glutaraldehyde sites, sterile glycine (0.5 mL of 1 M stock in PBS) is added and the beads mixed thoroughly for 1 hr. The antigen-conjugated beads are then washed three times and suspended in a final volume of 1 mL PBS. Beads can be stored as a suspension at 4°C.
2. Binding assay: Add 200 μL of PBS containing 5mg/mL BSA into a 1.5 mL microfuge tube. To this add 5.0 μL of antigen-coated bead suspension followed by 500 μL of BSA/PBS solution containing up to 100 ng of the radio-labeled antibody. Pipette an equal amount of radiolabeled antibody into gamma counter vials to serve as a standard.
3. Incubate the mixture on a tube rotator for 1 hr at RT.
4. To quantify the amount of bound mAb, the beads are isolated by centrifugation at $10,000 \times g$ for 2 min and the supernatant aspirated and discarded in an appropriate radiation waste stream.
5. Resuspend the beads thoroughly in 500 μL of PBS containing 0.1% Tween20 by pipetting. Repeat steps 4 and 5. 6. The specific activity associated with the beads can be measured in the microfuge tube by directly inserting it into a scintillation vial, or they can be resuspended and pipetted into an appropriate counting vial.

Active preparations of the 11-1F4 mAb yield binding of > 60% to the beads. Using this protocol and a peptide antigen of 30 amino acids the bead capacity appears to be much greater (theoretically 1000 fold) than that required to bind 100 ng of 11-1F4. We have not observed any change in %-bound fraction over the range of 11-1F4 mAb concentrations that we have tested (up to 1 μ g mAb). One sample of 125 I-labeled antibody stored at 4° C for over two weeks retained 65% binding in this assay.

4 Radiolabeling using the Succinimidyl-iodobenzoate (SIB) method

This method produces radio iodinated peptides or proteins that are resistant to dehalogenases found in the serum and liver. When compared to oxidative methods SIB labeling is slightly slower and usually produces proteins with 5-10 fold lower specific activities. A detailed procedure follows:

1. The starting material is not commercially available at this time. Trimethyl Sn 4 succinimidyl benzoate, MeATE, was synthesized using the method of Koziorowski (Koziorowski et al., 1998). The product was dried extensively and stored desiccated at -20 °C. Product stored at room temperature loses significant activity for iodide incorporation within months even if it is completely dry.
2. Prepare a 10 mg/mL stock solution of MeATE in methanol with 5% acetic acid (v/v). This stock solution can be stored for about a month at -20 °C.
3. Immediately prior to labeling, dilute a small amount the stock solution 1:50 into methanol with 5% acetic acid and transfer 5 μ L (~ 1.0 μ g) to a clean microfuge tube. Add radioactive iodide in dilute NaOH (up to 30 μ L or 3 mCi). Add 5 μ L of chloramine T (freshly prepared as described above).
4. To remove unbound iodide, add 0.5 mL of freshly prepared sodium metabisulfite solution and transfer to the barrel of a 5 mL plastic syringe with a C18 light SEPPAK cartridge (Waters, Milford, MA) attached. It is prudent to prime the cartridge by rinsing with methanol and then water before use.
5. Insert the syringe plunger and slowly push the iodinated mixture through. Remove the cartridge (carefully) and then remove the plunger from the syringe barrel. Add 3 mL of pure water to the reassembled cartridge barrel and wash it through slowly.
6. Remove the cartridge and plunger again and add 0.5 mL 100% methanol to the cartridge assembly. Slowly push the solution through and collect the iodinated tracer in a clean glass tube. Dry the eluant under a stream of nitrogen.
7. Redissolve the material with 100-200 μ L of analytical grade acetonitrile, transfer to a plastic microfuge tube and again evaporate to dryness. A quick pass with a Geiger-Müller detector should reveal >50% of the activity in this fraction.
8. To the dried, iodinated SIB, add 5 μ L of 1 M sodium borate solution pH 8.6-9.0. Add protein as a concentrated solution in PBS and allow the reaction to proceed for 15 min at RT. This step is limiting and low concentration proteins give lower yields.
9. Quench any remaining reaction by addition of 5 μ L of 1 M glycine in sterile-filtered PBS.
10. The coupled material can then be purified in open size exclusion gel filtration columns as described above. NOTE: Gelatin must be used as the carrier protein as BSA will bind contaminating glycy-SIB and carry it through the gel filtration process into the final product.

5 Injections

The most reproducible route of injection is intravenous (iv) – in mice, we use the lateral tail vein. Mice are exposed to a heat lamp for 5 minutes before restraining them in a plastic tube that allows exposure of the tail. One-mL plastic syringes are used with 27 gauge needles. Volumes up to 0.4 mL of neutral buffered, standard ionic strength solutions (such as PBS) can be injected without complications. Care must be taken to avoid air bubbles in the delivered volume. For our 11-1F4 mAb and SAP studies, 0.2 mL of radio iodinated antibody (10-50 µg, ~ 300 µCi) is injected in about 5 seconds. A gauze pad is used to apply pressure at the injection site and blot return blood before the animals are returned to their cages. In rare instances that the tail vein is not accessible, intraperitoneal (ip) injections can be used. We have found that small volumes (0.2 – 0.5 mL) of radio iodinated antibodies injected ip equilibrate in the circulation within about 2 hrs. Thus ip injection may be used for imaging in some cases; however, biodistribution of iv and ip injected animals can not be compared in the same groups even at later time points.

V Generating Murine Models of AA and AL Amyloid Disease

1 AL amyloidoma

There are presently no transgenic murine models of systemic AL amyloidosis and attempts to recapitulate the disease in mice have been successful but costly in terms of the substantial amounts of human light chain protein required. We have therefore developed a model of localized AL amyloidosis in mice which can be used to assess the efficacy of novel therapeutic agents and amyloidophilic radiotracers in vivo. Human (hu)AL amyloid was extracted and purified from amyloid-laden organs harvested at autopsy using the water floatation method of Pras (Pras et al., 1968). To prepare the amyloid for injection, lyophilized human AL amyloid extract was suspended in 25 ml of sterile PBS and homogenized with a PCU-2 Polytron apparatus at the highest setting for 3 minutes (Brinkman, Luzerne, Switzerland). The fibrils were sedimented by centrifugation at 6°C for 30 min at ~12000 × g and 50 to 100 mg of the resultant pellet was re-suspended in 1 ml of sterile PBS and rehomogenized using the PCU-2 Polytron, as above. Typically, two groups of three 8-wk old Balb/c mice (Charles Rivers, Wilmington, MA) are injected sub-cutaneously with amyloid solution using an 18 gauge needle. All 6 mice receive 40-100 mg of amyloid between the scapulae in a 1-2 mL volume of sterile phosphate-buffered saline (PBS: 150 mM NaCl, 5 mM NaHPO₄, Na₂PO₄). The amyloidoma, which was readily palpable and visible by microCT imaging, remained resident at the site of injection and became vascularized within 7 days post-injection (Fig. 2). The first group of mice received ¹²⁵I-11-1F4 mAb and the second group, serving as controls, was injected with ¹²⁵I-MOPC-31c, an isotype matched murine IgG1k mAb.

2 Induction of AA-amyloidosis in hIL-6 Transgenic Mice (TRIAD)

Systemic AA amyloidosis induced in mice has been successfully imaged by whole body planar scintigraphy following administration of ¹²³I- or ¹³¹I-labeled SAP (Caspi et al., 1987; Hawkins et al., 1988b) and with ¹¹¹In or ¹²⁵I-labeled AA-reactive rat monoclonal antibodies (Marshall et al., 1986). Although these studies demonstrated highly specific tracer uptake in hepatosplenic AA-amyloid deposits the anatomic resolution was insufficient to discern liver and spleen independently. Advances in microimaging hard- and software capabilities now enable dual-modality (SPECT/CT), high resolution images of amyloid-laden viscera in mice to be acquired, using ¹²⁵I-labeled SAP. Using these techniques we have demonstrated an excellent correlation between the biodistribution and quantitation of the amyloid deposits using ¹²⁵I-SAP imaging and standard histological evaluation (Wall et al., 2005a).

For imaging studies, AA amyloidosis is induced in mice carrying the human interleukin-6 (hIL-6) transgene, expressed constitutively under control of the murine major

histocompatibility complex (H2-L^d) promoter or metallothionein (MT) promoter (furnished by Dr. Michael Potter (Kovalchuk et al., 2002) and Dr. Ciliberto (Fattori et al., 1994), respectively). AA-amyloidosis is induced in 8-12 wk-old MT or H2/IL-6 mice by administering, 10 µg of amyloid enhancing factor (AEF, prepared as previously described (Axelrad et al., 1982; Kindy and de Beer, 1999) intravenously in the tail vein, in a volume of 100 µL sterile filtered PBS. At 6-wks post AEF-injection the amyloid deposits were visualized by dual SPECT/CT imaging using ¹²⁵I-labeled SAP as the tracer. AA amyloidosis can be induced in wild type mice by injection of AEF in conjunction with a pro-inflammatory stimulus such as silver nitrate solution (Snow and Kisilevsky, 1985), casein (Kindy and de Beer, 1999) or lipopolysaccharide (Inoue and Kisilevsky, 1999). NOTE: Sub-cutaneous silver nitrate solution can attenuate x-rays in the CT scan and would presumably also diminish the gamma emission from the ¹²⁵I-SAP or 11-1F4 mAb, although the potential effects of this on SPECT image quality have not been determined.

VI MicroSPECT and CT Image Acquisition

Thyroid uptake of free iodide in the AA and AL amyloid mice is restricted by adding Lugol's solution (1% v/v) to the drinking water 48 hrs prior to the administration of ¹²⁵I-labeled tracer. Mice with systemic AA amyloidosis receive an *iv* injection of 200 µL of ¹²⁵I-SAP solution in PBS (10-15 µCi/µg protein) in the lateral tail vein. The mice are sacrificed 24 hr. post-injection by overdose of isoflurane inhalation or carbon dioxide asphyxiation and placed in 50 mL centrifuge tubes and are then frozen before SPECT and CT scanning. Freezing is not necessary but when many mice are to be scanned it is prudent to freeze them as the acquisition of SPECT and CT data can take ~ 1 hr per animal.

For mice bearing subcutaneous AL amyloidomas, ~ 300 µCi of ¹²⁵I-labeled 11-1F4 mAb (~ 12 µCi/µg protein) is administered in the lateral tail vein. When mAbs are used the mice are sacrificed 72 hrs post injection to allow adequate clearance of the unbound reagent. The mice are sacrificed and frozen prior to collection of SPECT and CT images.

SPECT images were acquired by positioning the animal between the SPECT detector heads near the center of rotation. For these studies, hexagonal lead foil parallel hole collimators with 1.2 mm diameter, 27 mm long holes and 0.2 mm septa were employed. These collimators are very similar to clinical Low Energy High Resolution (LEHR) collimators. The collimators were placed within a few millimeters of the animal, providing approximately 2.5 mm resolution with 55 cps/MBq sensitivity. Each SPECT data set consisted of 60 projections acquired over a period of 60 seconds per projection.

The animals were transferred to the microCT scanner immediately following the SPECT data acquisition. The microCT system was configured for relatively high speed (~6 minute), low resolution (~100 micron) image acquisition. For these studies, the x-ray source was biased at 80 kVp with 500 µA anode current. The x-ray detector was configured for 4 × 4 binning, reducing the size of each projection to 512 × 768 pixels. Projections were acquired in 1-degree steps over a 360 degree orbit.

VII Image Co-registration

Our current imaging protocol utilizes independent microSPECT and CT machines which necessitates that we co-register the two image sets i.e., align them so that the anatomic CT image is correctly positioned with reference to the SPECT image. To date, we have found that this is most readily achieved manually using a 3-D visualization and analysis software package (Amira, Version 3.1: Mercury Computer Systems, Chelmsford, MA). Each SPECT and CT image contains fiducial markers supplied by capillary tubes filled with a solution of ¹²⁵I. Aligning the fiducials visible in 3-D reconstructions of each image ensures correct co-

registration of the nuclear and anatomic images. This process will be circumvented by the new dual-modality microSPECT/CT machine which will acquire both sets of image data without the mouse being moved – application of a simple mathematical transformation will result in accurately co-registered images in this instance.

VIII Image Reconstruction

1 CT Image Reconstruction

When dealing with high-count projection data as is the case here, a volumetric CT image is conveniently reconstructed using a filtered backprojection based method such as the algorithm of Feldkamp et al. (Feldkamp et al., 1984). Under ideal circumstances a ramp filter will suffice, but in practice a filter is used that contains some degree of builtin smoothing to prevent undesirable, high-frequency noise from being amplified. Typical filter choices include Shepp-Logan and Generalized-Hamming (Jain, 1989). Due to the divergent nature of a circular-orbit cone-beam x-ray system, filtered backprojection methods are capable of delivering perfect reconstructions of the center slice of the image whereas off-center slices are only approximate. However, this does not tend to be a problem unless the cone-angle is very large, i.e., in slices at the periphery of the image. In order to achieve reconstruction times on the order of 5-10 minutes, we employ parallel computing as well as a problem reducing technique called focus of attention (Gregor et al., 2002). The latter allows us to separate the projection data as well as the image data into “mouse” and “background” prior to reconstruction so that we subsequently concentrate the available computational resources solely on the “mouse” data.

2 SPECT Image Reconstruction

SPECT projection data tends to be count-poor and noisy. Use of an iterative image reconstruction method is therefore necessary. The goal is to solve a linear system of equations, $\mathbf{Ax} = \mathbf{y}$ where vectors $\mathbf{x}$ and $\mathbf{y}$ denote the unknown image and the acquired projection data, respectively, and $\mathbf{A}$ is a so-called system matrix that describes the geometry of the system plus detector efficiencies if the projection data is not pre-corrected. While an algebraic method could be used to solve this system in a weighted least squares sense or otherwise, it is often better to recast it in a statistical context and use a maximum-likelihood technique such the EM algorithm by Shepp and Vardi (Shepp and Vardi, 1982). Depending on how the system matrix is configured, count preservation can be achieved meaning that each iteration redistributes activity within the image without altering the overall photon count resulting in truly quantitative images. The main disadvantage of the EM algorithm is that it is slow to converge. While parallel computing could be used to speed up convergence, ordered-subsets constitute a much simpler solution (Hudson and Larkin, 1994). We have found that about 8-10 iterations using 4 subsets are sufficient to produce images of the quality shown in this paper. The time required to perform these calculations is about one minute on a standard laptop (Gregor et al., 2004).

3 Image Visualization and Analysis

For the purposes of 3-D image visualization and analysis, the raw data output from the microCT and SPECT image reconstructions are loaded into the Amira software package. The microCT and SPECT images can be represented in 3-D space as volumetric or surface views that can be readily co-registered using a manual transformation tool. The image data can be displayed as 2-D gray-scale slices in axial, sagittal or coronal aspects as well as in 3-D using a number of display modules. Additionally, both the 2-D and 3-D visualizations can be combined to generate complex representations of the data. Amira was used to generate the images presented in this chapter. Representative examples of microSPECT/CT images depicting the binding of ^{125}I -SAP to AA amyloid (Fig. 3) and ^{125}I -11-1F4 mAb to an AL amyloidoma (Fig. 4) in mice were generated using Amira.

IX Corroborative Studies: Biodistribution and Autoradiography

Although micro-imaging can produce excellent quantitative, high resolution data on the position of radioisotope in a live animal, studies on tissues gathered post mortem from the scanned animals can support and augment the imaging data. An effort is made to collect these data for all of our microSPECT experiments.

1 Biodistribution

There are many published methods to determine the organ distribution of injected radiotracers. To collect statistically meaningful data at least 5 mice per group are required. The animals should all be the same age and sex to eliminate differences due to size and weight. The animals are sacrificed by isoflurane inhalation or carbon dioxide asphyxiation, and microSPECT/CT data collected. The mice are either then dissected immediately after sacrifice (preferable) or after the images of animals frozen in 50 mL centrifuge tubes have been collected. Appropriate sized pieces of organs are placed into tared, plastic scintillation vials. The vials are tightly capped and weighed soon after organ collection to minimize weight loss to evaporation. Small organ samples should be taken from mice that have been injected with relatively large amounts of radioactivity (e.g. for microSPECT), so that counter efficiency is not compromised by coincidence counting of samples with high activity. Note: Cuts in the liver lead to leakage of blood into the peritoneal cavity which can be collected with a 200 μ L micropipette as a source of blood sampling. Alternatively, blood, serum or clotted blood can be collected from the thoracic cavity after removal of the heart lung block. Tissues should be dissected free of external blood clots and blotted on clean paper. If the mice are sacrificed using isoflurane or carbon dioxide inhalation and then frozen before the organs can be harvested it is difficult to collect blood samples and the mice often have lung hemorrhage which leads to anomalously high values of tracer accumulation in this organ. For most small soft tissue samples very little auto-quenching is noted for gamma scintillation counting even with soft gamma emitters (I-125). Internal standards for accurate counting of large bone samples may be necessary. Values for biodistribution are usually presented as % injected dose per gram of tissue (%ID/g). These values can easily be calculated from the net weight and net recovered counts per minute (cpm) relative to standards of the injected tracer solution set aside at the onset of the experiment. Including these standard samples when measurement of the experimental tissue samples is performed eliminates the need for isotopic decay correction. Figure 5 depicts representative biodistribution data collected from mice with AA amyloid deposits primarily in the spleen and liver with minor deposits visible in the kidney and heart. A control group of healthy mice is shown for comparison. Organs were harvested from both groups 24 hr post-injection of 125 I-SAP.

2 Autoradiography

Autoradiographs of tissue sections yield very high resolution micro distribution of radioactive probes (Fig. 6). Image analyses of silver grains can resolve radioactive decay, i.e., radiolabeled tracer accumulation, at the cellular level (Kennel et al., 1991). Thus, measurements of probe binding at the resolution of imaging (~ 50 μ m) or at the organ level from biodistribution studies can be refined to indicate the cell type or other target e.g. amyloid deposits that are the actual site of tracer accumulation. For amyloid imaging in the liver, spleen and kidneys, this is particularly useful to distinguish between actual amyloid uptake of radiolabeled probe and non-specific accumulation that can occur in these organs due to probe interaction with the reticuloendothelial cells or during renal tubular reabsorption. For this purpose it is necessary to compare the autoradiographic images with a Congo red stained tissue section (Congo red staining of amyloid is described in detail elsewhere in this series, Westermarck et al., 1999). Figures 6a and b are consecutively cut sections of a mouse liver containing significant quantities of AA amyloid. This is evidenced by the coincident localization of 125 I-SAP,

manifest by the appearance of black grains in the autoradiograph (Fig. 6a), with the amyloid deposits revealed by the characteristic green birefringence in the Congo-red stained section (Fig. 6b). The best autoradiographic images are obtained with I-125. Mice injected with ~300 μ Ci of tracer as in the microSPECT imaging experiments are ideal tissue sources. Tissues can be collected at the time biodistribution studies are done. Small tissue samples are fixed in 10% neutral buffer formalin for 1 day. The tissues are washed from the fixative into PBS and are then processed for paraffin embedding. Sections of 5 to 6 μ m thickness are cut and placed on Probond coated glass slides and air dried. In absolute darkness, the slides are dipped in NTB-2 liquid emulsion (Eastman Kodak, Rochester, NY) that has been warmed to room temperature. After drying the slides are stored in light tight boxes at room temperature for the incubation period. The time of exposure varies with the amount of radioisotope injected, the fraction accumulating at the target site and the density of grains desired. It is useful to make several copies of a test slide and develop them at various times to gauge the amount of exposure desired for the entire set – periods from 4-7 days are generally sufficient to provide excellent images. Slides are developed with Dektol (Eastman Kodak, Rochester, NY) and fixed with Kodak fixative, then the tissues are dehydrated for hematoxylin and eosin counterstaining and cover slip mounting. Slides prepared in this manner can be stored indefinitely until analyses can be done.

X Summary

Heretofore, evaluating and monitoring amyloid deposition in mouse models of disease has relied for the most part upon post mortem histological examination to determine organ involvement and the extent of amyloid deposition. As an alternative we have developed protocols for 125 I-labeling of SAP and the amyloid-binding mAb 11-1F4 that, in conjunction with high-resolution SPECT and CT imaging, provide a means to visualize systemic AA and localized AL amyloid deposits in mouse models of the disease. In conjunction with micro-autoradiographic and biodistribution studies the SPECT and CT images, when visualized using state of the art software, can be used to quantitate and determine of the extent of organ-specific amyloid deposition.

Techniques and imaging hard- and software we have described offer new opportunities not only to observe *ante mortem* and in real time the progression of amyloidosis *in vivo* but also a way to evaluate preclinically and in individual animals the efficacy of novel therapeutic agents designed to prevent or cure amyloid diseases.

Acknowledgements

The authors would like to thank all the people who have contributed to the protocols presented herein, in particular; Rudi Hrnac, Denny Wolfenbarger, Maria Schell, Trish Smith and Tina Richey for their tireless work with the mouse models. Trish Lankford and Justin Baba at Oak Ridge National Laboratories for their assistance with biodistribution measurements and image acquisition. Jim Wesley, Sallie Macy and Craig Wooliver for processing and staining of the mouse tissues and Philip Hawkins for his encouragement of this work and the gift of human SAP. AS is an American Cancer Society Professor. This work was supported by NIBIB Bioengineering Research Partnership Award # RO1 EB000789.

References

- Abe M, Goto T, Kennel SJ, Wolfenbarger D, Macy SD, Weiss DT, Solomon A. Production and immunodiagnostic applications of antihuman light chain monoclonal antibodies. *Am J Clin Pathol* 1993a;100:67–74. [PubMed: 7688492]
- Abe M, Goto T, Wolfenbarger D, Weiss DT, Solomon A. Novel immunization protocol and ELISA screening methods used to obtain and characterize monoclonal antibodies specific for human light chain variable-region subgroups. *Hybridoma* 1993b;12:475–83. [PubMed: 8244419]
- Auf dem Brinke D, Kohrle J, Kodding R, Hesch RD. Subcellular localization of thyroxine-5-deiodinase in rat liver. *J Endocrinol Invest* 1980;3:73–6. [PubMed: 7373011]

- Axelrad MA, Kisilevsky R, Willmer J, Chen SJ, Skinner M. Further characterization of amyloid-enhancing factor. *Lab Invest* 1982;47:139–46. [PubMed: 7109539]
- Bailey GS. Labeling of peptides and proteins by radioiodination. *Methods Mol Biol* 1994;32:441–8. [PubMed: 7951743]
- Behr TM, Gotthardt M, Becker W, Behe M. Radioiodination of monoclonal antibodies, proteins and peptides for diagnosis and therapy. A review of standardized, reliable and safe procedures for clinical grade levels kBq to GBq in the Gottingen/Marburg experience. *Nuklearmedizin* 2002;41:71–9. [PubMed: 11989301]
- Bellotti V, Mangione P, Merlini G. Review: immunoglobulin light chain amyloidosis--the archetype of structural and pathogenic variability. *J Struct Biol* 2000;130:280–9. [PubMed: 10940232]
- Bishop GM, Robinson SR. Physiological roles of amyloid-beta and implications for its removal in Alzheimer's disease. *Drugs Aging* 2004;21:621–30. [PubMed: 15287821]
- Bolton AE, Hunter WM. The labelling of proteins to high specific radioactivities by conjugation to a 125I-containing acylating agent. *Biochem J* 1973;133:529–39. [PubMed: 4733239]
- Caspi D, Zalzman S, Baratz M, Teitelbaum Z, Yaron M, Pras M, Baltz ML, Pepys MB. Imaging of experimental amyloidosis with 131I-labeled serum amyloid P component. *Arthritis Rheum* 1987;30:1303–6. [PubMed: 3689465]
- Cummings JL. Alzheimer's disease. *N Engl J Med* 2004;351:56–67. [PubMed: 15229308]
- De Beer FC, Pepys MB. Isolation of human C-reactive protein and serum amyloid P component. *J Immunol Methods* 1982;50:17–31. [PubMed: 7086148]
- De Nardo GL, De Nardo SJ, Miyao NP, Mills SL, Peng JS, O'Grady LF, Epstein AL, Young WC. Non-dehalogenation mechanisms for excretion of radioiodine after administration of labeled antibodies. *Int J Biol Markers* 1988;3:1–9. [PubMed: 3249042]
- Fattori E, Della Rocca C, Costa P, Giorgio M, Dente B, Pozzi L, Ciliberto G. Development of progressive kidney damage and myeloma kidney in interleukin-6 transgenic mice. *Blood* 1994;83:2570–9. [PubMed: 7513204]
- Feldkamp LA, Davis LC, Kress JW. Practical cone-beam algorithm. *J Opt Soc Am* 1984;1:612–619.
- Garg S, Garg PK, Zhao XG, Friedman HS, Bigner DD, Zalutsky MR. Radioiodination of a monoclonal antibody using N-succinimidyl 5-iodo-3-pyridinecarboxylate. *Nucl Med Biol* 1993;20:835–42. [PubMed: 8241995]
- Ghetti B, Tagliavini F, Takao M, Bugiani O, Piccardo P. Hereditary prion protein amyloidoses. *Clin Lab Med* 2003;23:65, viii–85. [PubMed: 12733425]
- Gregor, J.; Gleason, SS.; Kennel, SJ.; Paulus, MJ.; Benson, T.; Wall, JS. Proc. IEEE Nuclear Science Symposium and Medical Imaging Conference. Rome, Italy: 2004. Approximate volumetric system models for microSPECT; p. 4
- Gregor J, Gleason SS, Paulus MJ, Cates J. Fast Feldkamp reconstruction based on focus of attention and distributed computing. *International Journal of Imaging Systems and Technology* 2002;12:229–234.
- Hawkins PN. Serum amyloid P component scintigraphy for diagnosis and monitoring amyloidosis. *Curr Opin Nephrol Hypertens* 2002;11:649–55. [PubMed: 12394612]
- Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB. Specific localization and imaging of amyloid deposits in vivo using 123I-labeled serum amyloid P component. *J Exp Med* 1988a;167:903–13. [PubMed: 3351437]
- Hawkins PN, Myers MJ, Epenetos AA, Caspi D, Pepys MB. Specific localization and imaging of amyloid deposits in vivo using 123I-labeled serum amyloid P component. *J Exp Med* 1988b;167:903–13. [PubMed: 3351437]
- Hawkins PN, Myers MJ, Lavender JP, Pepys MB. Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component. *Lancet* 1988c;1:1413–8.
- Hnatowich DJ. Recent developments in the radiolabeling of antibodies with iodine, indium, and technetium. *Semin Nucl Med* 1990;20:80–91. [PubMed: 2404343]
- Hrncic R, Wall J, Wolfenbarger DA, Murphy CL, Schell M, Weiss DT, Solomon A. Antibody-mediated resolution of light chain-associated amyloid deposits [In Process Citation]. *Am J Pathol* 2000;157:1239–46. [PubMed: 11021828]

- Hudson HM, Larkin RS. Accelerated image reconstruction using ordered subsets of projection data. *IEEE Transactions of Medical Imaging* 1994;13:601–609.
- Hull RL, Westermark GT, Westermark P, Kahn SE. Islet amyloid: a critical entity in the pathogenesis of type 2 diabetes. *J Clin Endocrinol Metab* 2004;89:3629–43. [PubMed: 15292279]
- Inoue S, Kisilevsky R. In situ electron microscopy of amyloid deposits in tissues. *Methods Enzymol* 1999;309:496–509. [PubMed: 10507044]
- Jain, AK. *Fundamentals of Digital Image Processing*. Pearson Prentice-Hall; 1989.
- Karonen SL. Developments in techniques for radioiodination of peptide hormones and other proteins. *Scand J Clin Lab Invest Suppl* 1990;201:135–8. [PubMed: 2244182]
- Kennel SJ, Falcioni R, Wesley JW. Microdistribution of specific rat monoclonal antibodies to mouse tissues and human tumor xenografts. *Cancer Res* 1991;51:1529–36. [PubMed: 1997194]
- Kindy MS, de Beer FC. A mouse model for serum amyloid A amyloidosis. *Methods Enzymol* 1999;309:701–16. [PubMed: 10507056]
- Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA. Arresting amyloidosis in vivo using small-molecule anionic sulphonates or sulphates: implications for Alzheimer's disease. *Nat Med* 1995;1:143–8. [PubMed: 7585011]
- Kisilevsky R, Szarek WA, Ancsin JB, Elimova E, Marone S, Bhat S, Berkin A. Inhibition of amyloid A amyloidogenesis in vivo and in tissue culture by 4-deoxy analogues of peracetylated 2-acetamido-2-deoxy-alpha- and beta-D-glucose: implications for the treatment of various amyloidoses. *Am J Pathol* 2004;164:2127–37. [PubMed: 15161647]
- Kohrle J, Rasmussen UB, Ekenbarger DM, Alex S, Rokos H, Hesch RD, Leonard JL. Affinity labeling of rat liver and kidney type I 5'-deiodinase. Identification of the 27-kDa substrate binding subunit. *J Biol Chem* 1990;265:6155–63. [PubMed: 2318852]
- Kovalchuk AL, Kim JS, Park SS, Coleman AE, Ward JM, Morse HC 3rd, Kishimoto T, Potter M, Janz S. IL-6 transgenic mouse model for extraosseous plasmacytoma. *Proc Natl Acad Sci U S A* 2002;99:1509–14. [PubMed: 11805288]
- Koziorowski J, Henssen C, Weinrich R. A New Convenient Route to Radioiodinated N-succinimidyl 3- and 4-Iodobenzoate, Two Reagents for Radioiodination of Proteins. *Appl. Radiat. Isot* 1998;49:955–959.
- MacRaid CA, Stewart CR, Mok YF, Gunzburg MJ, Perugini MA, Lawrence LJ, Tirtaatmadja V, Cooper-White JJ, Howlett GJ. Non-fibrillar components of amyloid deposits mediate the self-association and tangling of amyloid fibrils. *J Biol Chem* 2004;279:21038–25. [PubMed: 15031287]
- Marshall, J.; McNally, W.; Muller, D.; Meincken, G.; Fand, I.; Srivastava, SC.; Atkins, H.; Wood, DD.; Gorevic, PD. In vivo radioimmunodetection of amyloid deposits in experimental amyloidosis. In: Glenner, GG.; Osserman, EF.; Benditt, EP.; Calkins, E.; Cohen, AS.; Zucker-Franklin, D., editors. *Amyloidosis*. Plenum Press; NY: 1986. p. 163-174.
- McConahey PJ, Dixon FJ. A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy Appl Immunol* 1966;29:185–9. [PubMed: 4160044]
- Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003;349:583–96. [PubMed: 12904524]
- Mihara M, Shiina M, Nishimoto N, Yoshizaki K, Kishimoto T, Akamatsu K. Anti-interleukin 6 receptor antibody inhibits murine AA-amyloidosis. *J Rheumatol* 2004;31:1132–8. [PubMed: 15170926]
- O'Neill, B.; Murphy, CL.; Wolfenbarger, DA.; Kennel, SJ.; Solomon, A.; Wall, JS. The Amyloid-Reactive Monoclonal Antibody 11-1F4 Binds a Cryptic Epitope on Fibrils and Partially Denatured Immunoglobulin Light Chains and Inhibits Fibrillogenesis. In: Grateau, G.; Kyle, RA.; Skinner, M., editors. *Amyloid and Amyloidosis: Proceedings of the Xth International Symposium on Amyloidosis*. CRC Press; Tours, France: 2005. p. 482-484.
- Paulus MJ, Gleason SS, Easterly ME, Foltz CJ. A review of high-resolution X-ray computed tomography and other imaging modalities for small animal research. *Lab Anim (NY)* 2001;30:36–45. [PubMed: 11385756]
- Paulus MJ, Gleason SS, Kennel SJ, Hunsicker PR, Johnson DK. High resolution X-ray computed tomography: an emerging tool for small animal cancer research. *Neoplasia* 2000a;2:62–70. [PubMed: 10933069]

- Paulus, MJ.; Gleason, SS.; Sari-Sarraf, H.; Johnsnosn, DK.; Foltz, CJ.; Austin, DW.; Easterly, ME.; Michaud, EJ.; Dhar, MS.; Hunsicker, PR.; Wall, JS.; Schell, M. BIOS 2000 International Biomedical Optics Symposium. San Jose: 2000b. High-resolution x-ray CT screening of mutant mouse models.
- Paulus MJ, Sari-Sarraf H, Gleason SS, Bobrek M, Hicks JS, Johnson DB, Behel JK, Thompson LH, Allen WC. A new x-ray computed tomography system for laboratory mouse imaging. *IEEE Trans. Nucl. Sci* 1999;46:558–564.
- Pepys MB, Booth DR, Hutchinson WL, Gallimore JR, Collins PM, Hohenester E. Amyloid P component. A critical review. *Amyloid: Int. J. Exp. Clin. Invest* 1997;4:274–295.
- Pras M, Schubert M, Zucker-Franklin D, Rimon A, Franklin EC. The characterization of soluble amyloid prepared in water. *J Clin Invest* 1968;47:924–33. [PubMed: 5641627]
- Rocken C, Shakespeare A. Pathology, diagnosis and pathogenesis of AA amyloidosis. *Virchows Arch* 2002;440:111–22. [PubMed: 11964039]
- Shepp LA, Vardi Y. Maximum-likelihood reconstruction for emission tomography. *IEEE Transactions on Medical Imaging* 1982;1:113–122.
- Snow AD, Kisilevsky R. Temporal relationship between glycosaminoglycan accumulation and amyloid deposition during experimental amyloidosis. A histochemical study. *Lab Invest* 1985;53:37–44. [PubMed: 2409350]
- Solomon A, Weiss DT, Wall JS. Immunotherapy in systemic primary (AL) amyloidosis using amyloid-reactive monoclonal antibodies. *Cancer Biother Radiopharm* 2003a;18:853–60. [PubMed: 14969598]
- Solomon A, Weiss DT, Wall JS. Therapeutic potential of chimeric amyloid-reactive monoclonal antibody 11-1F4. *Clin Cancer Res* 2003b;9:3831S–8S. [PubMed: 14506180]
- Spanka M, Hesch RD, Irmischer K, Kohrle J. 5'-Deiodination in rat hepatocytes: effects of specific flavonoid inhibitors. *Endocrinology* 1990;126:1660–7. [PubMed: 2307124]
- Stenstad T, Husby G, Brefeldin A inhibits experimentally induced AA amyloidosis. *J Rheumatol* 1996;23:93–100. [PubMed: 8838515]
- Vaidyanathan G, Affleck D, Welsh P, Srinivasan A, Schmidt M, Zalutsky MR. Radioiodination and astatination of octreotide by conjugation labeling. *Nucl Med Biol* 2000;27:329–37. [PubMed: 10938466]
- Vaidyanathan G, Zalutsky MR. Protein radiohalogenation: observations on the design of N-succinimidyl ester acylation agents. *Bioconjug Chem* 1990a;1:269–73. [PubMed: 2096920]
- Vaidyanathan G, Zalutsky MR. Radioiodination of antibodies via Nsuccinimidyl 2,4-dimethoxy-3-(trialkylstannyl)benzoates. *Bioconjug Chem* 1990b;1:387–93. [PubMed: 2099187]
- Wall, J.; Schell, M.; Hrnac, R.; Macy, SD.; Wooliver, C.; Wolfenbarger, DA.; Murphy, C.; Donnell, R.; Weiss, DT.; Solomon, A. Treatment of Amyloidosis using an anti-fibril monoclonal antibody: Preclinical efficacy in a murine model of AA-amyloidosis. In: Bely, M., editor. *Amyloid and Amyloidosis: Proceedings of the IXth International Symposium on Amyloidosis*. David Apathy; Budapest, Hungary: 2001. p. 158-160.
- Wall JS, Kennel SJ, Paulus MJ, Gleason S, Gregor J, Baba J, Schell M, Richey T, O'Nuallain B, Donnell R, Hawkins PN, Weiss DT, Solomon A. Quantitative High-Resolution Microradiographic Imaging of Amyloid Deposits in a Novel Murine Model of AA-Amyloidosis. *Amyloid: The Journal of Protein Folding Disorders*. 2005aIn press
- Wall, JS.; Paulus, MJ.; Kennel, SJ.; Gleason, S.; Baba, J.; Gregor, J.; Wolfenbarger, DA.; Weiss, DT.; Solomon, A. Radioimaging of Primary (AL) Amyloidosis with an Amyloid-Reactive Monoclonal Antibody. In: Grateau, G.; Kyle, RA.; Skinner, M., editors. *Amyloid and Amyloidosis: Proceedings of the Xth International Symposium on Amyloidosis*. CRC Press; Tours, France: 2005b. p. 37-39.
- Westermarck GT, Johnson KH, Westermarck P. Staining methods for identification of amyloid in tissue. *Methods Enzymol* 1999;309:3–25. [PubMed: 10507013]
- Wilbur DS. Radiohalogenation of proteins: an overview of radionuclides, labeling methods, and reagents for conjugate labeling. *Bioconjug Chem* 1992;3:433–70. [PubMed: 1463775]
- Zhu H, Yu J, Kindy MS. Inhibition of amyloidosis using low-molecular-weight heparins. *Mol Med* 2001;7:517–22. [PubMed: 11591887]

Figure 1. Schematic representation of the microCT imaging hardware. The X-ray source and CCD camera are fixed to the stage which can rotate 360° around the stationary mouse placed upon the bed.

Figure 2.

The murine model of localized AL amyloidoma. A) The human AL amyloidoma is introduced between the scapulae of Balb/c mice and is readily palpable (mouse is shaved to show the position of the amyloidoma). B) The amyloidoma appears vascularized within 7 days post injection (skin is removed to show subcutaneous vascularization around amyloid. The amyloidoma is readily visible by microCT imaging. C) 3-dimensional volume rendering of a mouse with a sub cutaneous amyloidoma. D) Axial CT slice through mouse shown in B at the level of the upper abdomen and amyloidoma. The mouse imaged in C and D was administered blood pool contrast medium (Fenestra VC™, Alerion Biomedical, San Diego, CA) iv prior to scanning. Arrows indicate the amyloidoma.

Figure 3.

Standard representations of microSPECT and microCT images obtained from ^{125}I -SAP labeling of AA-amyloid deposits in H2/IL-6 mice. A) Planar, 2-D, scintigraphic image of a mouse with accumulation of ^{125}I -SAP in the spleen (arrow) and to a lesser degree in the liver. B) Axial slice of a co-registered microSPECT/CT through the upper abdomen showing significant activity in the spleen (arrow) and diffuse accumulation of the SAP in the ventral liver. C) 3-D volume-texture (vortex) rendering of the microSPECT data discriminates the high amyloid content in the spleen (arrow) from the more diffuse hepatic deposits. D) Precise anatomic localization of the amyloid is provided by when a 3-D vortex rendering of the ^{125}I -SAP distribution is displayed with a surface rendered microCT dataset.

Figure 4.

Anatomic localization of a human AL amyloidoma. A) The amyloidoma is readily visible (arrow) and anatomically pinpointed when a 3-D surface rendering of the ¹²⁵I-11-1F4 mAb distribution is displayed with the CT image. B) When a ¹²⁵I-labeled isotype-matched control antibody (MOPC-31c IgG1κ; Sigma, St Louis MO) is used, no activity is observed in the amyloidoma. However, free iodide is visible in the thyroid (arrow). In both A and B a threshold was applied to the SPECT data to display the 40% maximal activity.

Figure 5.

Typical biodistribution data following ^{125}I -SAP injection into mice with systemic AA amyloidosis. H-2/IL-6 mice (with amyloid) and wild type, healthy mice (WT) received an iv injection of ^{125}I -SAP, were sacrificed 24 hr post-injection, and the organs harvested for analysis. The majority of the activity is observed in the spleen and liver with minor ($< 10\%$ ID/gram) amounts found in the kidneys and heart. No activity was associated with any of the organs evaluated from the WT mice

Figure 6.

Representative micro-autoradiographic study of splenic tissue from an AA amyloidotic H2/IL-6 mouse administered ^{125}I -SAP for SPECT imaging. Consecutive sections were stained using photographic emulsion (A) and alkaline Congo red (B). In (A), dense areas of amyloid appear as dark punctate regions whereas more diffuse accumulation of ^{125}I -SAP is manifest as shadowed areas. The amyloid deposits in (B) appear in cross-polarized illumination as green/blue birefringent areas. Magnification was $\times 40$ (5 $\times$ objective).