Senile seminal vesicle amyloid (SSVA), one of the most common forms of localized amyloidosis, is associated with the male aging process. Although it had been posited that the amyloidogenic component originated from exocrine cells and that, on the basis of immunohistochemistry, that the amyloid was composed of lactoferrin, the nature of SSVA was never established definitively. To address this issue, we have used our microanalytic techniques to characterize the structure of the congophilic green birefringent protein extracted from 5 such amyloid-containing specimens. Mass spectrometric analysis revealed that in all cases, the fibrils were composed mainly of polypeptide fragments identical in sequence to the N-terminal portion of the major secretory product of seminal vesicles, namely semenogelin I (SgI). Although lactoferrin was detected in 3 instances, the trace amount and seemingly intact form of this molecule indicated that it was not the amyloidogenic molecule. The SgI nature of the amyloid was confirmed through demonstration that the deposits were immunostained specifically with SgI-reactive antibodies. The results of our research provide unequivocal evidence that SSVA is derived from SgI, and we provisionally designate this form of amyloidosis as ASgI. (J Lab Clin Med 2005;145:187–93)

Abbreviations: ABTS = 2,2’-azino-di-3-ethyl-benzthiazoline sulfonic acid; AEC = 3-amino-9-ethyl-carbozol; HPLC = high-performance liquid chromatography; mAb = monoclonal antibody; MS-MS = tandem mass spectrometry; PAP = peroxidase-antiperoxidase; SDS/PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SgI = semenogelin I; SSVA = senile seminal-vesicle amyloid
In contrast to the systemic amyloidoses, where the pathology is typically found within blood-vessel walls, SSVA is confined exclusively to the seminal vesicles. Evidence indicating that the congophilic material was derived from an endogenous protein was obtained by Cornwell et al, who found that an antiserum raised against a 14-kD component extracted from vesicular deposits reacted specifically with SSVA, as well as with seminal-vesicle epithelium. On this basis, these investigators posited that the fibrillar protein was unique and that it represented the first example of an amyloidogenic precursor of exocrine cell origin. Subsequently, others claimed, solely on the basis of immunohistochemical findings, that SSVA was formed from lactoferrin. Although the protein identity of amyloid may be inferred with the use of this technique, it is important to note that the results obtained with this method sometimes are misleading or inconclusive due to false-positive reactions or the lack of suitable antisera. We therefore have emphasized that to determine unequivocally the nature of the fibrils, it is necessary that they be isolated and characterized by amino-acid sequencing and/or mass spectrometry.

In this regard, we have applied our microextraction and analytic techniques to establish the exact chemical composition of SSVA. We analyzed 5 such cases and report here that in each instance, the amyloid was derived from the major secretory product of the seminal-vesicle epithelium, namely Sgl. Furthermore, the pathologic deposits were immunostained specifically with antibodies directed against Sgl-related protein.

**METHODS**

**Clinical specimens.** Fresh frozen or formalin-fixed, paraffin-embedded specimens of seminal vesicles were obtained from 5 men ranging in age from 62 to 87 years. Samples were derived at the time of surgery from a patient with prostate cancer (L-13) and during autopsy from the other 4 individuals (Am61, Am62, 2521, and 2004). Amyloid was detected in all 5 cases after Congo red staining and examination under polarizing and fluorescence microscopy.

**Amyloid extraction and chemical characterization.** Amyloid fibrils were extracted from 4-μm-thick formalin-fixed, paraffin-embedded tissue sections using 6 mol/L guanidine hydrochloride as described by Murphy et al and from frozen tissue in accordance with the water-solubilization method of Pras et al. After reduction and carboxymethylation, the proteins were purified by reverse-phase HPLC on an Aquapore 300 C8 (30 × 4.0 mm) column (Brownlee Columns; Perkin Elmer, Norwalk, Conn) using a linear aqueous 0.1% trifluoroacetic acid–7% acetonitrile to a 0.1% trifluoroacetic acid–70% acetonitrile gradient at a flow rate of 1 mL/min; the fractions corresponding to the UV-absorbing peaks were collected manually and dried using a Speed Vac (Savant Instruments, Farmingdale, NY). For digestion with trypsin (Promega, Madison, Wis), protein fractions were reconstituted with 20 μL of 100 mmol/L ammonium bicarbonate buffer (pH 8) containing 0.4 μg of enzyme. The digested samples were loaded into an autosampler (Surveyor; Thermofinnigan, Thousand Oaks, Calif), and 5 μL of each was injected into an LC-Packings Vydac RP-18 0.3 × 150-mm column (Dionex, Sunnyvale, Calif). Chromatography was performed using a Surveyor pump at a rate of 60 μL/min with the output split 30:1 to send a volume of 1.5 to 2 μL/min to the column. A gradient of 5% to 90% acetonitrile in 0.1% formic acid was developed over the course of 40 minutes. The eluent from the column was directed through a nanospray into an LCQDeca ion trap mass spectrometer (Thermofinnigan). Instrument control and peptide identification were performed using the manufacturer’s software programs Xcalibur and Bioworks, respectively.

**Proteins.** Purified Sgl, isolated from semen, was provided by Dr Hans Lilja and Dr Johan Malm (Lund University Hospital, Malmö, Sweden), and seminal plasma was provided by Dr Hans Wolf (University Hospital of Dermatology, Munich, Germany). Lactoferrin was purchased from Sigma-Aldrich (Steinheim, Germany).

**Antibodies.** Antisera were raised in rabbits by methods previously described, using purified Am61 amyloid and thyroglobin coupling 22- and 20-mer Sgl-related synthetic peptides corresponding to positions 1 through 22 and 22 through 41, respectively, of the mature protein (composition and purity were established by mass spectrometry, courtesy of Dr Georg Arnold, Genzentrum, Munich, Germany). A murine mAb directed against the C-terminal 30 residues of human Sgl was furnished by Dr Hans Lilja and Dr Johan Malm. Rabbit anti–human lactoferrin was purchased from Sigma-Aldrich.

**SDS-PAGE and Western blotting.** SDS-PAGE was performed on 12.5% polyacrylamide gels using a sample buffer containing 0.1 mol/L β-mercaptoethanol and 0.2% SDS. The separated proteins were stained with Coomassie brilliant blue R250 and, from a parallel gel, transferred to nitrocellulose (B85; Schleicher & Schüll, Dassel, Germany) by capillary blotting in 0.1 mol/L sodium bicarbonate (pH 11.2). Calibrated molecular-weight standards (Blue R Plus2) were obtained from Invitrogen (Karlsruhe, Germany). Nitrocellulose-immobilized proteins were identified immunologically using the PAP system and the reaction visualized with ABTS (Sigma-Aldrich).

**Immunohistochemical studies.** Deparaffinized 4-μm-thick sections were incubated with the polyclonal antisera (diluted 1:2000–4000) or mAb (1:10–50) and washed, after which immunoreactivity was visualized using the PAP method. Color was developed with AEC (Sigma-Aldrich).