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Primary localized amyloidosis of the eyelid: two cases of immunoglobulin light chain-derived proteins, subtype λV respectively λVI

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SUMMARY

Primary localized amyloidosis has been described in many different organs in the body. Studies by immunohistochemical techniques have suggested an immunoglobulin light chain origin of the amyloid material. Only in a limited number of cases has the amyloid protein been characterized by amino acid sequence analysis as subtypes of immunoglobulin light chain or heavy chain. In this report, two cases of primary localized amyloidosis of the eyelid are presented. The amyloid substance has been extracted and a major fibril protein subjected to amino acid sequence analysis. Both amyloid proteins were part of the variable region of immunoglobulin light chains, subtype λV and subtype λVI, respectively. While λVI has been shown to be a common subtype in systemic immunoglobulin light chain-amyloidosis, it has never been demonstrated in localized amyloid. Very few λV immunoglobulin light chains have been characterized and the subgroup has never been found in amyloid before.

Keywords amyloid conjunctiva immunoglobulin lambda

INTRODUCTION

Primary localized amyloidosis (PLA) without evidence of systemic amyloid disease has been found in almost every organ in the body. The symptoms and the significance for the patient depend on the site of location and on the amount of amyloid deposited: an aggregation of amyloid in the brain can give rise to epileptic seizures; amyloid in the larynx can cause airway obstruction; nodular amyloidosis in the lung may not give any clinical symptoms, but causes diagnostic problems differentiating it from neoplasms.

In most reported cases of PLA, the occurrence of amyloid substance has been shown histochemically with alkaline Congo red stain giving green birefringence in polarized light, but attempts to further characterize the amyloid has been done only in a limited number of patients. In most cases immunohistochemical techniques have been applied and suggestions of immunoglobulin light chain origin of the amyloid material have been deduced from the positive-staining reaction of the amyloid substance and/or the surrounding plasma cells. In 12 cases of PLA, amino acid sequence analysis of extracted amyloid fibrils has been performed giving evidence of immunoglobulin origin, light chain type in 11 cases and heavy chain type in 1 case (Table 1) [1–12]. There has been no suggestion of any other protein constituents.

The occurrence of amyloid in the eye and in the eye adnexal structures is well known, but uncommon. It can be found as part of a systemic amyloidosis or as a localized phenomenon. It is important for the patient to state if the amyloidosis is systemic or localized, because systemic amyloidoses influence life expectancy, but localized amyloidosis gives symptoms locally and can often be controlled by excision. Most of the cases reported in the literature deal with the findings of amyloid itself (symptoms, treatment, differential diagnoses). In a few reports immunohistochemical stainings of the amyloid material and/or the surrounding plasma cells indicate an immunoglobulin origin of the amyloid (Table 2) [12–15]. In one case amino acid sequence analysis of extracted amyloid fibrils from the orbit gave evidence of immunoglobulin heavy chain [12].

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MATERIALS AND METHODS

Case 1

G.D., a 49-year-old woman, was first seen in 1987 with ptosis of the left upper eyelid for 3 months. A firm mass was located in the upper lid extending from the medial to the lateral canthus. Orbital computed tomography scanning showed no orbital extension. A conjunctival biopsy demonstrated amyloidosis (Fig. 1). Medical and laboratory evaluation including rectal biopsy was negative for systemic amyloidosis. The patient was followed until 1992 when a resection of the subconjunctival mass was performed.

Case 2

A.J., a 65-year-old woman, was referred to an ophthalmologist in 1986 for a right lower eyelid swollen for 5 months. A soft tumour

Table 1. Localized immunoglobulin-derived amyloid forms in which the major fibril protein has been characterized by amino acid sequence analysis

Reference	Site	Immunoglobulin
Husby *et al*. [1]	Cutan, nodular	λI
Westermark *et al.* [2]	Larynx	KI or III
Sletten *et al*. [3]	Cutan, nodular	KIII
Kitajima *et al*. [4]	Cutan, nodular	λIII
Linke *et al*. [5]	Cerebrum	λ
Vidal *et al*. [6]	Cerebrum	λ II or V
Berg *et al*. [7]	Larynx	KI
Eriksson *et al*. [8]	Cerebrum	λIV or III
Miura *et al*. [9]	Lung	λIII
Vigushin *et al*. [10]	Extradural space*	KIV
Eilam *et al*. [11]	Nasopharynx""	λIII
Tan *et al*. [12]	Orbita	γ1 or γ4

* Localized lymphoma.
"" Plasmacytoma.

measuring approximately 10 × 15 mm was present in the inferotemporal palpebral conjunctiva. The tumour was removed surgically and a histopathological examination showed amyloid. In 1992 there was recurrence of a mass in the lower fornix and palpebral conjunctiva at the same site which was excised. There was no clinical evidence of primary or secondary amyloidosis. There was no history and no clinical or laboratory evidence of inflammatory, renal or cardiac disease. A biopsy from the rectal mucosa and a punch biopsy of the skin of the thigh did not show amyloid deposits. The patient was and in 1995 still is in good health.

Table 2. Characterization of localized amyloid in the eye lid and orbita, immunohistochemically or by amino acid sequence analysis

Reference	Location	Results
Lucas *et al*. [13]	Orbita	Plasma cells with λ and IgA
	Eye lid	Plasma cells with κ and IgG
Schaldenbrand and Keren [14]	Eye lid	Plasma cells with λ and IgD Amyloid stained for IgD
Borodic *et al*. [15]	Subconjunctival	Amyloid stained for κ and λ
Tan *et al*. [12]	Orbita	γ1 or γ4

Purification of amyloid fibril protein

From both cases, small amyloid-laden tissue pieces were stored at −20°C until extraction of fibrils. This was performed mainly according to the method of Pras *et al*. [16] with the modification that the pellet material was used for purification of amyloid fibril proteins. From Case 1, about 20 mg of lyophilized crude amyloid fibril preparation was obtained, while in Case 2 only 5 mg of material were available. In both instances, the fibril material was defatted in chloroform : methanol (2 : 1) and dissolved overnight in 6 M guanidine HCL in 0·1 M Tris HCl buffer, pH 8·0, containing 0·1 M ethylene diamine tetra-acetic acid (EDTA) and 0·1 M dithiothreitol. After centrifugation, the supernatant was applied to a

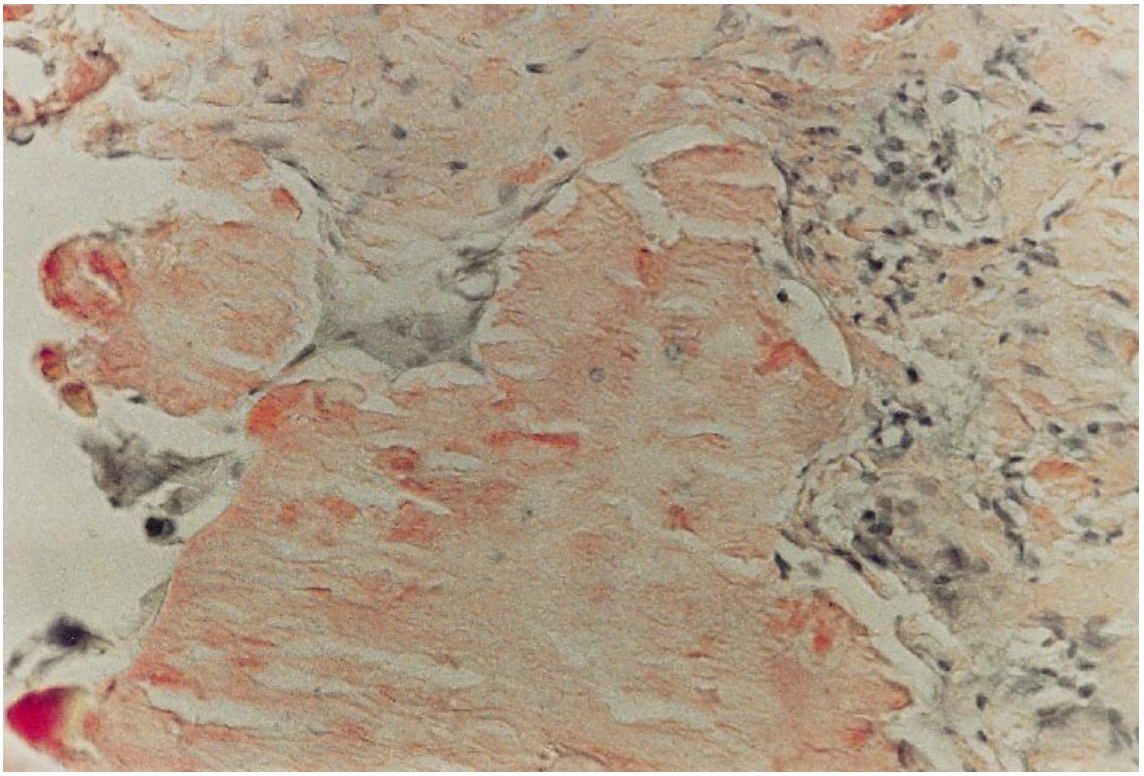

Fig. 1. Amyloid in the eye lid stained with Congo red; ×400.

Sepharose 6B CL column (Pharmacia, Uppsala, Sweden), and equilibrated with 5 M guanidine HCl in distilled water which also was used for elution. After gel filtration, pooled fractions corresponding to major peaks were precipitated with saturated ammonium sulphate, dialysed exhaustively against deionized water and lyophilized.

Material (7 mg) from Case 1 was redissolved in 5 M guanidine HCl containing 0·1 M dithiothreitol and rerun on a Sephacryl S300 HR column (Pharmacia), other conditions being as for the first gel filtration. Fractions corresponding to the main retarded peak were pooled, precipitated, dialysed and lyophilized as described above.

Material from Case 2 was dissolved in 5 M guanidine HCL while still in the lyophilization vessel and injected onto a Brownlee 30 × 4·6 mm C_4 reversed phase column (Applied Biosystems, San Jose, CA) connected to an LKB HPLC apparatus (Bromma, Sweden). Bound material was eluted with a linear gradient of 70% acetonitrile in 0·1% trifluoroacetic acid and analysed at 226 nm.

Analytic procedures

SDS 10–20% gradient PAGE was performed according to the method of Blobel and Dobberstein [17].

Amino acid sequence analysis

An automatic protein sequencer (477A, Applied Biosystems, Perkin Elmer, Foster City, CA) coupled to a PTH amino acid analyser, was used for N-terminal amino acid sequence analysis. Cleavage with BNPS-skatole was performed as described earlier [18].

RESULTS

In both instances sections of the affected eyelids showed heavy deposits of amyloid in the subepithelial tissue. There was also a moderate infiltration of lymphocytes and plasma cells (Fig. 1). A trial to characterize the plasma cells according to immunoglobulin light chain subtype did not give a conclusive result.

From both cases amyloid fibrils were extracted and a major fibril protein purified by gel filtration. In Case 1, sufficient material was obtained for a second gel-filtration step. Analysis of the purified amyloid fibril protein peak material by SDS–PAGE revealed several low molecular weight bands, the predominating one with a molecular mass of about 17 kD (Fig. 2). An N-terminal amino acid sequence analysis run in 38 steps gave a sequence typical of a λVI immunoglobulin light chain, positions 1–37 (Fig. 3). The same material was then taken for cleavage with BNPS-skatole and re-applied to the sequencer. The sequence revealed positions 38–49.

In Case 2, only very little protein material was available and therefore the protein from the initial gel filtration was subjected to reverse-phase high-performance liquid chromatography (RP-HPLC), which gave rise to two distinctive but closely eluted peaks. N-Terminal analysis of peak 1 revealed an amino acid sequence starting at position 67 of an immunoglobulin light chain and comprising about 0·8 nmol (Fig. 4). Analysis of peak 2 material, run for nine steps, also gave the same sequence, also with a yield of 0·8 nmol. In addition, in peaks 1 and 2 materials a sequence corresponding to an immunoglobulin light chain starting at position 3 was established which in both cases comprised about 0·2 nmol. The first residues could not be identified. In peak 2 material, 0·5 nmol of a sequence starting at position 111 was also identified. The sequences obtained indicate a λV immunoglobulin light chain. However, in this case the N-terminal residue, pyrolidone carboxylic acid (Pca), seen in several λ subgroups, was missing [19].

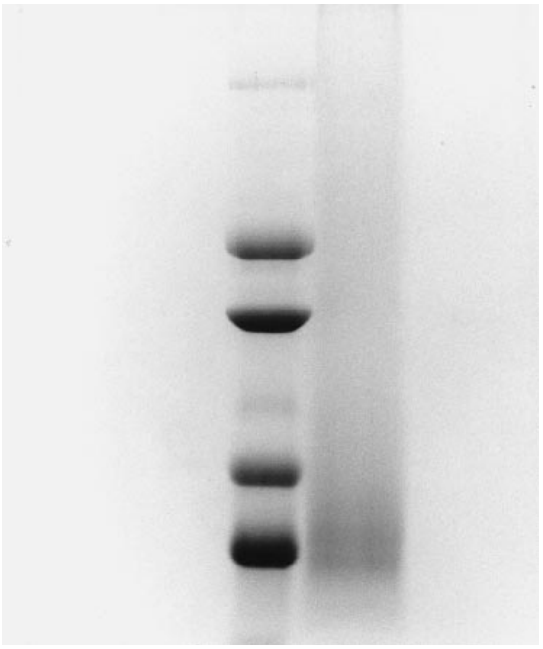

Fig. 2. SDS–PAGE of amyloid fibril material from Case 1 (right lane). Standard molecular markers (left lane) are, from top: ovalbumin (46 kD), carbonic anhydrase (30 kD), trypsin inhibitor (21·5 kD), lysozyme (14·3 kD).

DISCUSSION

Although not completely proved, the immunoglobulin light chain deposited as localized AL-amyloidosis is most likely to be produced close to the deposition site. That means that a plasma cell clone synthesizing this monoclonal protein must be present at this

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          1                   5                  10
AL-GD   (Asp)Phe-Met-Leu-Thr-Gln-Pro-His-Ser---Val-Ser-Glu-Ser-
AL-AR   ----------------------------------------------------

15                  20                  25           27A  27B
Pro-Gly-Gly-Thr-Val-Thr-Ile-Ser(Cys)Thr-Gly-Ser(Ser)(Ser)(His)-
--------Lys-------------Phe--------------------  Gly-  Gly-  Ser -

          30                  35                  40
Val-Xxx(Asp)Asn-Ser/Tyr-Val-Gln(Trp)Tyr-Gln-Gln(Arg)Pro-Gly-
Ile-Ala-Asp Ser  -Phe-   -----------------------------------

            45              49
Ser-Ala-Pro(Thr)Ser-Val-Ile-Tyr-  (Present case 1)
----------------Thr-------------  (24)
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Fig. 3. Amino acid sequence of a protein purified from amyloid fibrils of Case 1. The sequence is typical of an immunoglobulin light chain, subgroup λVI. Xxx, unidentified residue. Residues in parentheses were not completely verified. The numbering system is according to the method of Kabat *et al*. [19].

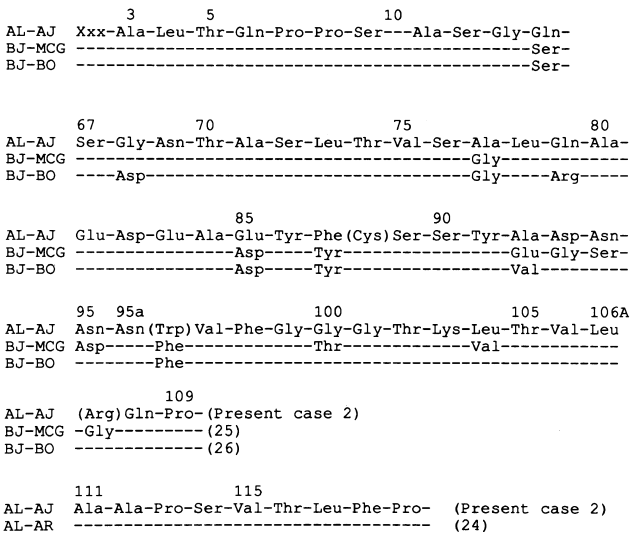

Fig. 4. N-terminal amino acid sequence of peptides purified from amyloid fibrils of Case 2, indicating an immunoglobulin light chain, subgroup λV. Three fragments were identified in the amyloid fibrils, the major starting at position 67. Two minor components started at position 3 and 111, respectively. Xxx, unidentified residue. Residues in parentheses were not completely verified. The numbering system is according to the method of Kabat *et al*. [19].

location. In fact, some degree of plasma cell infiltration is generally seen in localized AL-amyloid deposits. However, plasma cell clones of defined κ or λ subgroups have been difficult to identify since plasma cells producing both κ and λ immunoglobulin light chains usually are present in localized amyloid deposits.

Localized AL-amyloidosis is rare but has been described at many different anatomical sites including the urinary bladder, respiratory tract, brain and skin. Localized amyloid, which has been supposed to be of AL-nature, is known to occur in the conjunctiva. However, to the best of our knowledge, this is the first report to characterize this amyloid biochemically. As a matter of fact, in total only a few localized AL-amyloids have been studied biochemically, probably due to the problems involved in obtaining unfixed material, necessary for protein purification. Of these characterized cases that we have found in the literature, four were of κ and seven of λ types. No localized amyloids of proven λV or λVI types have been reported previously. Immunoglobulin light chain λVI is one of the most prevalent in systemic AL-amyloidosis [20], while no such case of ALλV has been found previously.

Amyloid fibrils may form as a result of off-pathway aggregation of intermediates during folding–unfolding [21]. The reason why only certain immunoglobulin light chains assemble into amyloid fibrils is not well understood. There is, however, evidence that the structure of the precursor protein determines its fibrillogenicity. Although all AL-proteins described hitherto have differed from each other in amino acid sequence, certain amino acid substitutions are significantly over-represented in AL-proteins compared with immunoglobulins that have not been associated with amyloid. Amino acid substitutions at some positions seem to be particularly important for fibrillogenesis [22].

Almost all AL-proteins are proteolytic cleavage products of immunoglobulin light chains and comprise the variable segment with a piece of the constant region. The length of the AL-protein varies, not only between AL-proteins but also in the deposits of a single individual, in that the AL protein material contains a mixture of fragments with somewhat different lengths. This is also true for the localized forms and in accordance with the findings in the present study in which SDS–PAGE of the major peak material of Patient 1 (ALλVI) showed several low molecular weight protein bands. Whether or not cleavage is of primary importance for fibrillogenesis is a matter of discussion. However, the virtually constant occurrence of such cleavage speaks in favour of proteolysis with removal of a part of the constant segment being a profibrillogenic event.

Analysis of Case 2 gave interesting and unusual results. While almost all AL-proteins described start N-terminally, the major fibril component in Case 2 was an immunoglobulin light chain fragment starting at position 67, while only minor amounts started N-terminally in position 3. According to Kabat *et al*. [19], Pca is the N-terminus in λV subgroup which was deleted in this case. The fragmentation pattern is somewhat different from most others, but it is possible that an unusual proteolytic cleavage has given rise to an amyloidogenic polypeptide, although, as stated above, the possibility of a post-fibrillogenic cleavage cannot be completely excluded. A second unusual finding was a fragment of the constant region, commencing with alanine 111. An identical fragment was found in amyloid HAR [23], a λII immunoglobulin light chain-derived protein associated with systemic amyloidosis. This cleavage in the constant region is trypticlike since position 110 is a lysine residue. It may be argued that systemic AL-amyloidosis was not completely excluded in our two patients since a small monoclonal plasma component could have escaped detection and since the patients were not studied by radioactive serum amyloid P component scan [27]. However, systemic amyloidosis is very unlikely given the long follow-up time without symptoms of the disease and the negative biopsy results in both patients.

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