Acta Dermatovener (Stockholm) 61: 381–384, 1981

AMYLOID IN LOCALIZED CUTANEOUS AMYLOIDOSIS: IMMUNOFLUORESCENCE STUDIES WITH ANTI-KERATIN ANTISERUM ESPECIALLY CONCERNING THE DIFFERENCE BETWEEN SYSTEMIC AND LOCALIZED CUTANEOUS AMYLOIDOSIS

Shin-ichi Masu, Michiko Hosokawa and Makoto Seiji

Department of Dermatology, Tohoku University School of Medicine
1-1 Seiryomachi, Sendai 980, Japan

Abstract. Amyloid of localized cutaneous amyloidosis and systemic amyloidosis were subjected to study with an indirect immunofluorescence technique using anti-keratin antiserum. Anti-keratin antiserum was prepared ad modum Sun & Green. Amyloid of localized cutaneous amyloidosis was positively stained for the antiserum, whereas amyloid of systemic amyloidosis (primary and multiple myeloma-associated) was negative. There was no difference between primary localized cutaneous amyloidosis (lichen amyloidosus and macular amyloidosis) and secondary localized cutaneous amyloidosis (amyloidosis associated with skin tumor). These results indicate that amyloid of localized cutaneous amyloidosis contains components derived from epidermal fibrous protein, probably tonofilaments of keratinocytes.

Key words: Keratin; Anti-keratin antiserum; Amyloid

Recently, the transitional forms between Civatte bodies (degenerated keratinocytes) and amyloid have been reported to be found in PUVA-treated human skin (2), in Riehl's melanosis (4, 5) and in primary localized cutaneous amyloidosis (3). It was speculated that Civatte bodies may be precursors of amyloid.

On the other hand, Sun & Green (8, 9) have extracted keratin proteins from the stratum corneum of human soles and produced the antiserum against the keratin proteins. In their indirect immunofluorescence studies with the antiserum, they described how the specific fluorescence was observed on the sites where tonofibrils are expected to be present in cultured human epidermal cells. In our previous papers (6, 7), it was shown that amyloid of primary localized cutaneous amyloidosis (PLCA) was positively stained for the anti-keratin antiserum which was prepared from the stratum corneum of human soles ad modum Sun & Green (8, 9). The result clearly indicates that amyloid of PLCA contains components derived from epidermal fibrous protein, which is consistent with the hypothesis that Civatte bodies may be precursors of amyloid of PLCA. However, the following suspicion may arise: the keratin protein may be secondarily adsorbed on amyloid as a result of the damage to keratinocytes after amyloid has been deposited in the dermis. In this report, comparative studies were performed on amyloids deposited in the skin in cases of systemic amyloidosis, so-called secondary localized cutaneous amyloidosis and PLCA in order to elucidate the pathogenesis of localized cutaneous amyloidosis.

MATERIALS AND METHODS

Skin specimens were obtained from patients with primary localized cutaneous amyloidosis (PLCA), secondary localized cutaneous amyloidosis (SLCA), primary systemic amyloidosis and systemic amyloidosis associated with multiple myeloma (Table 1). After biopsy of the skin

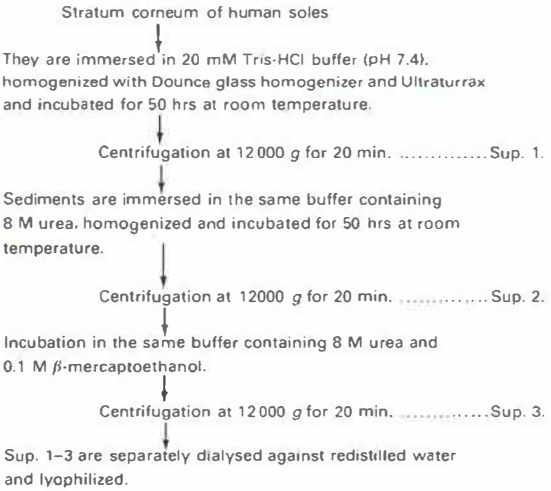

Fig. 1. Extraction procedures for keratin proteins.

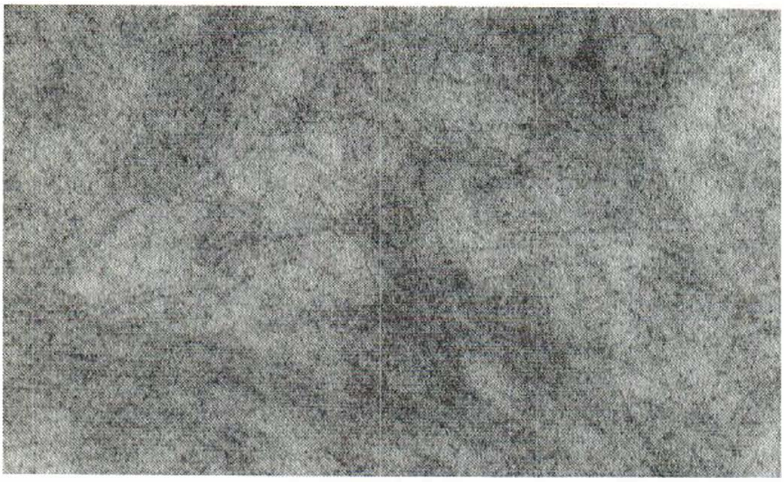

Fig. 2. Electron migrograph of amyloid of secondary localized cutaneous amyloidosis. Amyloid mass consists of straight and non-branching filaments. ×60 000.

lesion, the specimens were cut immediately into three pieces with sharp razor blades for routine histological study, immunofluorescence study and electron microscopic study. For the histological study, the specimens were fixed in a neutral formaldehyde solution, embedded in paraffin, and stained with hematoxylin-eosin, thioflavin-T, congo red and crystal violet. For the electron microscopic study, the specimens were fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide. After fixation, dehydration and embedding were performed according to a standard formula. The ultrathin sections were double-stained with uranyl acetate and lead citrate, and observed with an electron microscope (JEM 100 B). For the indirect immunofluorescence study, the specimens were immediately frozen at –80°C in a deep freezer and stored until use. Immunofluorescence staining was performed on 6 μm cryostat sections using standard techniques within 2 weeks after biopsy. Fluorescein-labelled goat anti-rabbit IgG (Miles Laboratories, F/P = 3.3) was applied at a dilution of 1 : 32. Specimens were observed under a Nicon fluorescence microscope (epi-illumination).

The extraction of keratin proteins and the production of anti-keratin antiserum were performed ad modum Sun & Green (8, 9). Briefly, the stratum corneum was obtained from normal human soles, and the extraction of keratin proteins was performed as shown in Fig. 1. Each extract obtained during step-by-step extraction processes was examined by SDS (sodium dodecyl sulfate) polyacrylamide gel electrophoresis (6, 7). Keratin proteins were obtained from sup-3 of Fig. 1. Antiserum against keratin proteins was produced with ordinary immunization techniques in New Zealand white rabbits. Double diffusion tests showed two precipitin bands between the keratin fraction and the antiserum, although the band on the antigenic side was very faint (6, 7). The keratin fraction did not react with the pre-immune rabbit serum. The antiserum was used at a dilution over 1 : 40.

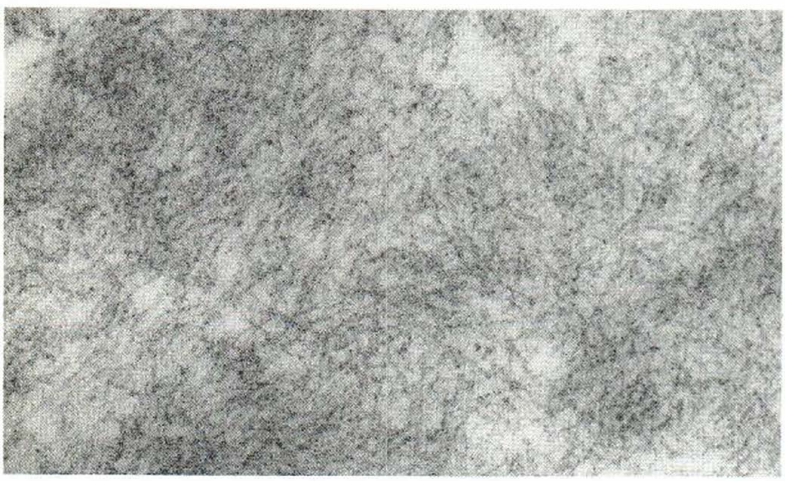

Fig. 3. Electromicrograph of amyloid of primary systemic amyloidosis. Filaments similar to those of secondary localized cutaneous amyloidosis can be observed. ×60000.

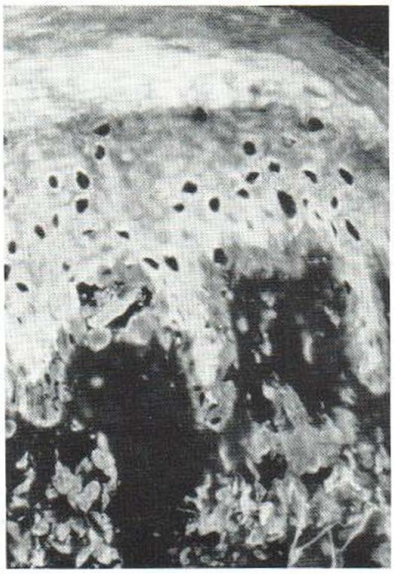

Fig. 4. Indirect immunofluorescent staining of primary localized cutaneous amyloidosis (lichen amyloidosus). Specific fluorescence is observed on the epidermis and amyloid masses.

Table 1. *Presentation of cases in this study*

	No. of cases
Primary localized cutaneous amyloidosis (PLCA)	
Macular amyloidosis	3
Lichen amyloidosus	1
Secondary localized cutaneous amyloidosis (SLCA)	
Bowen disease	1
Basal cell carcinoma	1
Giant porokeratosis	1
Systemic amyloidosis (SA)	
Primary SA	1
SA associated with multiple myeloma	1

RESULTS

The presence of amyloid in the dermis was ascertained in each case histologically and/or electron-microscopically. Amyloid masses of systemic amyloidosis were relatively larger than those of localized cutaneous amyloidosis. Although amyloid staining was negative in a few cases, the typical amyloid masses were confirmed in the electron microscope in these cases. Amyloid masses showed a characteristic filamentous structure in both localized and systemic amyloidosis, and these filaments were irregularly arranged (Figs. 2, 3). No difference between these amyloid masses could be discerned in the electron microscope.

In all specimens, the specific fluorescence for keratin proteins was intensely positive throughout the epidermis, from basal cells to stratum corneum except nuclei. Melanocytes and Langerhans cells appeared to be negative. There was no specific fluorescence on any components of the dermal stroma. In localized cutaneous amyloidosis (PLCA and SLCA), most amyloid masses were intensely stained for the anti-keratin antibody in all cases (Figs. 4, 5), although a few weakly or negatively

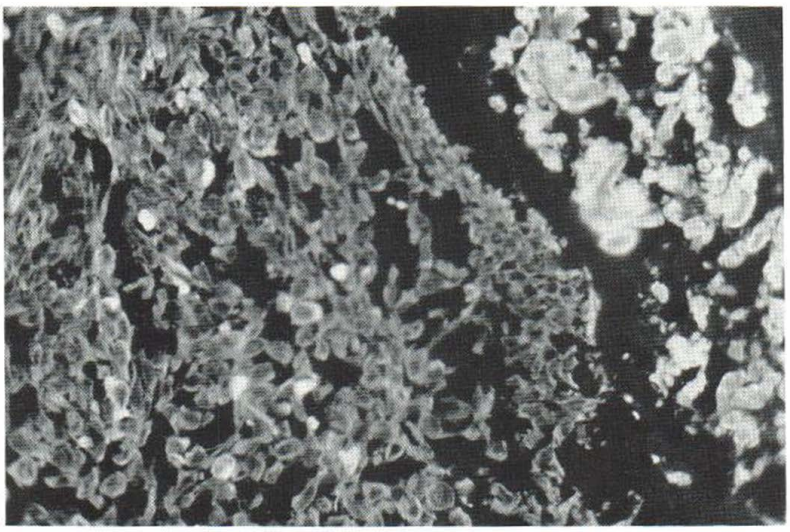

Fig. 5. Indirect immunofluorescent staining of secondary localized cutaneous amyloidosis associated with basal cell carcinoma with anti-keratin antiserum. Specific fluorescence is serum observed on tumor cells (left) and amyloid (right).

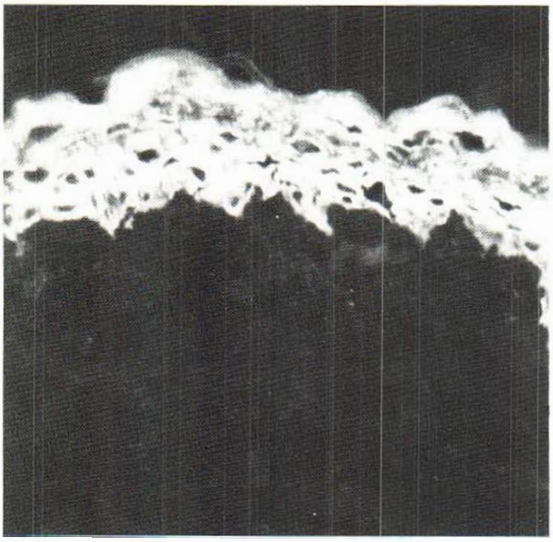

Fig. 6. Indirect immunofluorescent staining of systemic amyloidosis associated with multiple myeloma. Specific fluorescence is observed only on the epidermis.

stained masses were observed. On the other hand, amyloid masses were entirely negative in primary systemic amyloidosis and systemic amyloidosis associated with multiple myeloma (Fig. 6).

DISCUSSION

It is well known that there are at least two types of systemic amyloidosis—primary and secondary. Amyloid fibrils found in systemic amyloidosis associated with multiple myeloma are the same as those in the primary systemic type in its structural protein, that is, they consist of fragments of immunoglobulin light chains (AL protein) (1). On the other hand, amyloid fibrils in secondary systemic amyloidosis consist of another protein, protein AA (1). Although their structural proteins differ, they are identical in their ultrastructure (1). In the case of localized cutaneous amyloidosis, however, the structural protein has not yet been clarified, probably because of difficulties in extraction of amyloid from the dermis and purification. Recently, it was demonstrated by indirect immunofluorescence techniques using ani-keratin antiserum, that amyloid of PLCA (lichen amyloidosus and macular amyloidosis) possessed the same antigenicity as keratin protein or epidermal fibrous protein (6, 7). The present study further showed that amyloid of SLCA was the same in this resepct. On the other hand, amyloid of systemic amyloidosis (primary and associated with multiple myeloma) was entirely negative for the anti-keratin antiserum. Although we have not yet studied secondary systemic amyloidosis, it is now possible to assume that the keratin proteins do not secondarily deposit on amyloid in the case of localized cutaneous amyloidosis. These results are considered to support the hypothesis that amyloid of localized cutaneous amyloidosis is derived from the degenerated keratinocytes (Civatte bodies) which are composed of filaments derived from tonofilaments (2–5). The pathogenesis of SLCA and PLCA may be the same. There were a few amyloid masses which were weakly stained or unstained for the anti-keratin antiserum in localized cutaneous amyloidosis. The antigenicity as keratin may have been diminished in these masses, but further studies are necessary to confirm this hypothesis. Studies on localization of the anti-keratin antibody may resolve this problem and such studies are now in progress.

REFERENCES

1. Franklin, E. C.: Amyloid and amyloidosis of the skin. J Invest Dermatol *67:* 451, 1976.
2. Hashimoto, K. & Kumakiri, M.: Colloid-amyloid bodies in PUVA-treated human psoriatic patients. J Invest Deramtol *72:* 70, 1979.
3. Kumakiri, M. & Hashimoto, K.: Histogenesis of primary localized cutaneous amyloidosis. J Invest Dermatol *73:* 150, 1979.
4. Masu, S., Sato, A. & Seiji, M.: A case of lichen amyloidosus with Riehl's melanosis-like lesion on the face. J Dermatol *6:* 161, 1979.
5. Electron microscopic studies on Civatte body in Riehl's melanosis. Tohoku J exp Med *131:* 177, 1980.
6. Masu, S., Hosokawa, M. & Seiji, M.: Studies on cutaneous amyloidosis with anti-keratin antibody. Jpn J Dermatol *90:* 623, 1980. (In Japanese.)
7. Immunofluorescence studies on cutaneous amyloidosis with anti-keratin antibody. Tohoku J Exp Med *132:* 121, 1980.
8. Sun, T-T. & Green, H.: Keratin filaments of cultured human epidermal cells. J Biol Chem *253:* 2053, 1978.
9. Immunofluorescent staining of keratin fibers in cultured cells. Cell *14:* 469, 1978.

Received December 16, 1980

S. Masu, M.D.
Department of Dermatology
Tohoku University
School of Medicine
1-1 Seiryomachi
Sendai 980
Japan