Immunofluorescence Studies on Civatte Bodies and Dyskeratotic Cells with Anti-Keratin Antibody

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HOSOKAWA, M., MASU, S. and SEIJI, M. Immunofluorescence Studies on Civatte Bodies and Dyskeratotic Cells with Anti-Keratin Antibody. Tohoku J. exp. Med., 1981, 135 (3), 219–229 —— So-called Civatte bodies and dyskeratotic cells were investigated in some skin disorders by using indirect immunofluorescence techniques with anti-human keratin antibody. In the disorders with lichenoid tissue reaction such as lichen planus and DLE, Civatte bodies were observed in the lower epidermis and upper dermis and they reacted distinctly to the anti-keratin antibody. In malignant skin tumors which show dyskeratotic cells in the epidermis, such as basal cell epithelioma and Bowen’s disease, dyskeratotic cells were more clearly reacted to the antibody than other keratinocytes. These observations present additional new evidence for the hypothesis that Civatte bodies are derived from tonofilaments of keratinocytes. —— Civatte body; dyskeratotic cell; keratin

In many inflammatory skin disorders and epidermal neoplasms, eosinophilic bodies are frequently observed in the epidermis and dermis. They are called Civatte bodies and/or dyskeratotic cells. Under the electron microscope, in general, dyskeratotic cells contain densely aggregated tonofilaments, and most of them possess condensed nuclear remnants (Seiji and Mizuno 1969; Olson et al. 1969). They are frequently observed in skin tumors such as basal cell epithelioma and Bowen’s disease. Civatte bodies, on the other hand, consist of loosely aggregated filaments, and seldom possess nuclear remnants (Ebner and Gebhart 1972; Ishibashi et al. 1978; Masu et al. 1979, 1980a). Civatte bodies are frequently observed in the dermo-epidermal junction of the skin in the disorders which show the degeneration of the basal cell layer, such as lichen planus, DLE and Riehl’s melanosis (Pinkus 1973). Although it is now generally considered that Civatte bodies are derived from degenerated epidermal keratinocytes (Ebner and Gebhart 1972; Anton-Lamprecht and Tilgen 1973; El-Labban and Kramer 1974; Hashimoto 1976; Ishibashi et al. 1978; Masu et al. 1979, 1980a), there are some investigators who speculate that Civatte bodies may be derived from melanocytes (Hashimoto and Kumakiri 1979) or dermal cells (Medenica and Lorincz 1977). The nature of their structural proteins is not known well as yet.

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In this study, anti-keratin antibodies were made according to the method of Sun and Green (1978), and dyskeratotic cells and Civatte bodies were investigated in some skin disorders by the indirect immunofluorescence technique. In addition, direct immunofluorescence studies with anti-immunoglobulins and anti-C3 antiserum as well as electron microscopic studies were performed, and the origin and nature of Civatte bodies are discussed.

**MATERIALS AND METHODS**

The extraction of keratin proteins and the preparation of anti-keratin antibody were performed according to the method of Sun and Green (1978). The method for extraction of keratin is summarized in Fig. 1. The lyophilized sample of 8 M urea and β-mercaptoethanol extracts were used as keratin proteins in the following experiments. Protein concentration was determined by the method of Lowry et al. (1951).

SDS polyacrylamide gel electrophoresis was performed by the method of Fairbanks et al. (1971). Samples were heated at 100°C for 3 min in a buffer containing 10 mM Tris/HCl (pH 7.4), 2.5% SDS, 10% sucrose, 1 mM EDTA and 5% β-mercaptoethanol and layered on the 7.5% gel under a current of 7 mA each for 2 hr. Pharmacia low molecular weight calibration kit was used as standard proteins.

Keratin was dissolved in 8 M urea and β-mercaptoethanol and dialysed with Tris/HCl containing 5 mM dithiothreitol for 2 days, then negatively stained with 4% uranyl acetate and examined by an electron microscope (JEM 100B).

For the preparation of anti-keratin antibody, a total of 20 mg of protein of extracted keratin was emulsified with Freund adjuvant (complete adjuvant for primary and incomplete adjuvant for two subsequent immunization) and injected intradermally over a period of 2 months into New Zealand white rabbits at multiple sites including the foot pads. Prepared antiserum was examined with extracted keratin on Ouchterlony's double diffusion method in 1% agar with 50 mM sodium phosphate (pH 7.4) and 0.1% SDS.

Tissue specimens were biopsied from 5 cases of lichen planus (a 57-year-old male and 14, 35, 52 and 77-year-old females), 1 case of DLE (52-year-old female), 1 case of
prurigo pigmentosa (18-year-old female), 2 cases of basal cell epithelioma (79-year-old male and 45-year-old female), 2 cases of Bowen’s disease (60-year-old male and 76-year-old female), and 2 cases of nevus pigmentosus (73-year-old male and 29-year-old female). Skins were also obtained from newborn rats and guinea pigs. The samples were cut into three pieces and used each for histologic, immunofluorescence and electron microscopic studies.

For histologic studies each sample was fixed in 10% neutral formaldehyde solution and embedded in paraffin.

For immunofluorescence studies, samples were immediately frozen and stored at −80°C. For the indirect method, samples were cut in cryostats at −20°C and incubated with an over 1:40 dilution of anti-keratin antibody at 37°C for 30 min. Then they were washed with PBS (pH 7.2) and further incubated with a 1:32 dilution of FITC-anti rabbit IgG goat antiserum (Miles Laboratories) and washed again. For the direct method, FITC-anti human IgG, IgA, IgM and C3 rabbit antiserum (each 1:40 dilution) (Behringwerke) were used, and incubation and washing were carried out similarly. The sections were observed in a Nicon fluorescence microscope (epi-illumination).

In one case of lichen planus, the specimens were examined by indirect immunofluorescence, then the same specimens were fixed with ethanol and stained further with H-E stain.

For electron microscopic studies, each specimen was fixed in 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide. After fixing, dehydration and embedding were performed according to standard methods. The ultrathin sections were doubly stained with uranyl acetate and lead citrate, and observed in an electron microscope (JEM 100B).

**RESULTS**

On SDS polyacrylamide gel electrophoresis, keratin protein extracted with Tris/HCl-urea-β-mercaptoethanol showed four main bands, molecular weights of which were between 64,000 and 43,000 (Fig. 2). There were a few protein bands of higher molecular weight (more than 90,000) and a staining which did not enter the gel was observed. These results were in good accordance with those of Sun and Green (1978) (Masu et al. 1980b, c).

When the keratin solution was negatively stained and observed under the electron microscope, filamentous structures about 90 Å in diameter were recognized.

When Ouchterlony’s double diffusion method was carried out, one strong precipitin line was observed between extracted keratin and its antiserum. One more faint line was observed at the site of antigen. Keratin did not react with pre-immune serum (Masu et al. 1980b).

When the skin specimens from normal human, newborn rat and guinea pig were stained by the indirect immunofluorescence technique with the anti-keratin antibody ranging from 1:40 to 1:1280 in dilution, the entire epidermis was stained positive except for the stratum granulosum which was weakly positive. Also, very weakly stained dots were found which were assumed to be consistent with keratohyalin granules. The stratum corneum was stained homogeneously but the spinous layer was more reticular or heterogeneous because of distinct, negatively stained nuclei. Neither epidermal nor dermal melanocytes of nevus pigmentosus were stained with the anti-keratin antibody. When the anti-keratin antibody was preincubated with keratin proteins at 37°C for 1 hr and then the immunofluorescence staining was carried out, almost no fluorescence was seen.
In H-E stains of all cases of lichen planus, DLE and prurigo pigmentosa, homogeneous eosinophilic globular bodies (Civatte bodies) were observed at the dermo-epidermal junction (Fig. 3). These bodies were revealed to be negative to Congo red, thioflavin T or Crystal violet stain.

Electron microscopically, these bodies were composed of loosely aggregated electron lucent filaments and melanosomes. Vacuoles were also observed in them but they were small in number compared with those in dyskeratotic cells as stated below (Fig. 4).

In all cases of lichen planus, DLE, and prurigo pigmentosa investigated by the indirect immunofluorescence technique, homogeneously stained globular bodies were seen at the lower epidermis and upper dermis either solitarily or in clusters (Fig. 5). By the direct immunofluorescence of serial sections, deposition of IgM was detected in these bodies and some bodies were positively stained with anti-IgA and anti-C3 antisera. No distinct deposition of IgG was observed.

In the case of lichen planus, the same sections were stained with H-E after they were examined by indirect immunofluorescence microscopy. It was revealed that eosinophilic Civatte bodies exactly corresponded to the sites positively
reacted on anti-keratin antibody. Some of the small bodies positive with the antibody could not be detected on H-E stained sections, but there were no Civatte bodies which were recognized as eosinophilic bodies with H-E stain but were negative with anti-keratin antibody (Figs. 6, 7).

On the other hand, in basal cell epithelioma and Bowen's disease, dyskeratotic cells with eosinophilic cytoplasm and with or without pyknotic nuclei were seen in the neoplastic epidermis. These cells were, in electron microscopic observation, composed of densely aggregated tonofilaments mostly containing nuclear remnants. Many melanosomes and vacuoles were often observed in them.

By indirect immunofluorescence microscopy with anti-keratin antibody, homogeneous bodies which were stained more clearly than other keratinocytes were scattered in the epidermis of these skin tumors (Fig. 8). They seemed to be consistent with dyskeratotic cells.

Fig. 4. Electron micrograph of a Civatte body. Civatte body consists of wavyly-arranged fine filaments (×9,000). Inset: Enlargement of a part of Civatte body (×60,000).
Fig. 5. Indirect immunofluorescence with the anti-keratin antibody (×160) in lichen planus. Homogeneously stained round bodies are seen in the upper dermis. The epidermis is also positive with the antibody except its nuclei.

Fig. 6 (left). Indirect immunofluorescence with the anti-keratin antibody (×80) in lichen planus. Positively stained Civatte bodies are present in the upper dermis.

Fig. 7 (right). After indirect immunofluorescence study, the same specimen seen at Fig. 6 was fixed in ethanol and re-stained with H-E stain. Eosinophilic Civatte bodies can be detected at exactly the same sites where they are positive with the immunofluorescence (arrows). Many melanophages are observed around them.
Moreover, in a case of basal cell epithelioma and in 2 cases of Bowen's disease, homogeneous masses stained with the anti-keratin antibody in the upper dermis were also recognized (Fig. 9). In a case of basal cell epithelioma, these masses were positive with Congo red, thioflavin T and crystal violet stain. Electron microscopically, most masses consisted of straight and non-branching filaments showing the characteristics of the amyloid masses (Fig. 10), and a few in the dermo-epidermal junction demonstrated filamentous structures which were identified as Civatte bodies.
DISCUSSION

In cytological or histochemical studies on Civatte bodies, their origin and chemical nature have been controversial. Thyresson and Moberger (1957) examined Civatte bodies in the cases of lichen planus histochemically and demonstrated that they contained high concentrations of nucleoprotein of ribosomal type and sulfhydryl groups. They suggested that Civatte bodies may be epithelial cells containing intracytoplasmic viral inclusion bodies. Other histochemical investigations on lichen planus (Golts and Hult 1963) showed strongly PAS-positive Civatte bodies and suggested that they contained a glycoprotein or polysaccharide. Also, contrary to the results of Thyresson and Moberger (1957), they demonstrated that Civatte bodies were slightly positive with sulfhydryl group stain and contained neither RNA nor DNA. They denied the viral nature of Civatte bodies and further suggested that they may be derivatives of either epithelial or inflammatory cells.

On the other hand, in chronic erythematodes, Braun-Falco (1954) observed ovoid PAS-positive “Schollenbildung” in the subepidermal papillary regions and

Fig. 10. Electron micrograph of amyloid observed in the dermis of Bowen’s disease (×24,000). Amyloid consists of straight and non-branching filaments. These filaments are irregularly oriented and not as fine as those of Civatte bodies.
sought their origin from the PAS-positive, thickened basement membranes. Ueki (1969) examined several dermatoses histochemically and indicated the possibility of the presence of two kinds of Civatte bodies, that is, those from epidermal origin in cases of LE and lichen planus and those originated from thickened basement membranes in cases of LE and dermatomyositis.

Recent electron microscopic studies showed that Civatte bodies consist of a filamentous structure and it is generally accepted that they are derived from degenerated keratinocytes (Ebner and Gebhart 1972; Anton-Lamprecht and Tilgen 1973; El-Labban and Kramer 1974; Hashimoto 1976; Ishibashi et al. 1978; Masu et al. 1979, 1980a). However, there are still other hypotheses that state dermal as well as epidermal cells (Medenica and Lorincz 1977) or melanocytes (Hashimoto and Kumakiri 1979) may contribute to the formation of the Civatte body.

Until now only a little has been known about the structural proteins of Civatte bodies. Sümegi (1979) investigated Civatte bodies in some dermatoses with lichenoid tissue reaction histochemically and suggested that fibrinoid necrosis may appear in the Civatte bodies and that keratin and precursors are present in them.

Our present observation by the indirect immunofluorescence method demonstrated that Civatte bodies were fluorescent after treated with anti-keratin antibody, and that the whole epidermis was also stained positive except nuclei. This would support directly that Civatte bodies contain keratin in their structural proteins. Moreover, we could not detect any Civatte bodies which were positive with H-E stain but negative with anti-keratin antibody in the same specimens. As neither melanocytes nor dermal components were positive with anti-keratin antibody, it does not seem likely that Civatte bodies are derived from melanocytes or dermal components alone.

Our results of direct immunofluorescence studies where deposition of IgM was shown on the Civatte bodies were consistent with those reported by other investigators (Baart de la Faille-Kuyper and Baart de la Faille 1974; Abell et al. 1975; Varelzidis et al. 1979; Danno et al. 1980). In those studies, the epidermal basement zone or papillary vessels sometimes showed deposition of immunoglobulins or fibrin as well as Civatte bodies. In our study, none of the dermatoses examined revealed positive staining on basement membranes or dermal vessel walls except DLE, in which deposition of C3 was detected in the basement membrane zone. The discrepancies between the results of our studies and those of other investigators cannot be explained at present. The difference in the stages of lesions examined may be one of the factors.

In 3 cases of epidermal neoplasms, homogeneous bodies which were positive with the anti-keratin antibody were recognized in the adjacent upper dermis. Electron microscopically, most of these bodies showed the characteristics of amyloid masses, and a few in the dermo-epidermal junction demonstrated the features of Civatte bodies. Although little has been studied about the Civatte bodies associated with epidermal neoplasms, Sümegi (1979) reported histochemically that Civatte bodies which showed the same reaction as in lichen planus were
observed in epithelial skin cancer. Masu et al. (1980b, c) demonstrated that amyloid of primary localized cutaneous amyloidosis (lichen amyloidosis and macular amyloidosis) was positively stained with the anti-keratin antibody and speculated that Civatte bodies might be precursors of amyloid. Our study indicates the possibility of the presence of Civatte bodies in the skin tumors and the successive formation of tumor-associating amyloid. Though the detailed mechanism of the formation of Civatte bodies has not been clarified as yet, it is assumed that once dyskeratotic cells occur in the epidermis, they may be removed by a trans-epidermal elimination and, at the same time, some may be excluded into the dermis with or without lichenoid tissue reaction and become Civatte bodies. Our indirect immunofluorescence method with the anti-keratin antibody would be useful for clarifying the conditions around Civatte bodies in some dermatoses.

References


