Acta Derm Venereol (Stockh) 1990; 70: 194–198

IgA Immunoreactive Deposits Collocal with Fibrillin Immunoreactive Fibers in Dermatitis Herpetiformis Skin

KARIN DAHLBÄCK and LYNN SAKAI

Departments of Dermatology, [1]University of Lund, University Hospital, S-221 85 Lund, Sweden, and [2]Oregon Health Sciences University, Shriners Hospital for Crippled Children, Portland, Oregon 97201 USA.

The IgA immunoreactive granules or fibrils, characteristically found in dermal papillae of patients with dermatitis herpetiformis, were previously reported to be associated with microfibrillar bundles. Recently, fibrillin, a component of such 8–12 nm microfibrils, was identified. In normal skin, the fibrillin immunoreactive microfibrils are present at the periphery of elastic fibers and are also present without concomitant amorphous elastin in the dermal papillae close to the lamina densa.

The localization of the IgA immunoreactive material in the dermal papillae of 17 patients with dermatitis herpetiformis was compared with the distribution of the fibrillin immunoreactive fiber network. Immunofluorescence methods using FITC- and TRITC-labelled antibodies, an avidin- biotin-peroxidase complex technique, and standard elastin staining procedures, were used in several sequential and double staining procedures. In 13 specimens, in which the IgA reactivity was granular, most of the granules were located at the sites of fibrillin-reactive structures. As it could not be excluded that the collocality was coincidental, it could not be ascertained whether the IgA granules were in fact related to the fibrillin immunoreactive fibers in these specimens. However, in 4 specimens with both granular and fibrillar IgA immunoreactive deposits, these were clearly related to and located at the sites of fibrillin-reactive fibrils in the dermal papillae. The results confirm earlier reports of an association of IgA reactive deposits with microfibrillar bundles in dermatitis herpetiformis skin, though the possibility of their binding to other extracellular matrix component(s) has not been ruled out. The findings suggest that fibrillin may be the structural component (or one of them) to which IgA reactive deposits bind in the skin of patients with dermatitis herpetiformis. ***Key words: Dermatitis herpetiformis, IgA, fibrillin, microfibrils.***

(Accepted November 13, 1989.)

Acta Derm Venereol (Stockh) 1990; 70: 194–198.

K. Dahlbäck, Department of Dermatology, University of Lund, University Hospital, S221-85 Lund, Sweden.

A granular and sometimes fibrillar pattern of IgA- and C3- immunoreactivity in the tips of the dermal papilla, close to the dermal-epidermal junction, is a characteristic finding both in lesional and normal skin in patients with dermatitis herpetiformis (DH) (1–3). The pathogenesis of DH is still obscure, as is the role of complement and IgA in this disease. A relationship with celiac disease is indicated by signs of gluten-sensitive enteropathy in DH patients and by the beneficial effect obtained with a gluten-free diet (2, 4, 5).

The origin of the dermal IgA is unknown. It consists of IgA type 1. As both IgA_1 and IgA_2 are produced in the gut, it would seem that, unless there is a selective binding of IgA_1, dermal IgA derives from another source (6). Both types of IgA are present in the circulating immune complexes found in the sera of some DH patients, indicating that the dermal IgA is not derived from such immune complexes (6). IgA immunoreactive deposits have also been found in jejunal biopsies from patients with dermatitis herpetiformis and from patients with celiac disease (7). The findings of a study using extraction procedures have suggested that the IgA reactive deposits in the dermal papillae of patients with DH contain aggregated IgA (8). Immunoreactivity of vitronectin and C9 neoantigen has been demonstrated in such granules, suggesting presence of SC5b-9 terminal complement complexes in addition to C3 and IgA (9,10). Immunoreactivity of fibrin has also been reported in these deposits (11).

The nature of the structures to which the IgA-reactive deposits bind, is unclear. The granules are located close to the dermal epidermal junction, a region where anchoring fibrils and 10 nm microfibrils attach to the lamina densa. The microfibrils form a continuum with elastin-associated 10 nm microfibrils lower in the papillary dermis (12). IgA immunoreactivity has been demonstrated on amorphous substances in close association with such microfibrils near the dermal epidermal junction (11,

13–15). Association of the IgA-reactive deposits with anchoring fibrils has also been reported (15). The chemical composition of the 10 nm microfibrils is still unclear. Fibrillin, a 350 kD glycoprotein, was recently demonstrated in conjunction with elastin-associated microfibrils and other 10 nm microfibrils and is presumedly a constituent of such microfibrils (16, 17). The dermal fibrillin immunoreactive fiber probably has a structural function, connecting the amorphous elastin to the lamina densa and to the extracellular matrix (18). Close to the lamina densa microfibrillar bundles lack amorphous elastin, and the fibrillin-reactive fibrils in this region also lack vitronectin and amyloid P component immunoreactivity that are normally present at periphery of elastic fibers in adults (18–20).

The present study was designed to ascertain whether the dermal IgA deposits in dermatitis herpetiformis are associated with fibrillin fibers in the dermal papillae.

MATERIALS AND METHODS

The skin specimens studied were from 17 patients with clinical and immunohistochemical signs of dermatitis herpetiformis, and comprised surplus material from biopsies referred to the laboratory for diagnostic immunofluorescence examination from ten different hospitals in Sweden. They were from perilesional skin (7 specimens) or from normal skin (10 specimens). Only specimens from cases with typical clinical pictures were included in the study. Diagnostic immunofluorescence demonstrated granular C3 and IgA reactivity in the tips of dermal papillae in all the specimens studied and in four of the specimens there was additional fibrillar IgA reactivity with immunostaining of perpendicular fibers in the dermal papillae.

Fixation procedure

The specimens were immersed in a transport medium (550 g ammonium sulphate added to 1 liter of 25 mM potassium citrate, 5 mM N-ethylmaleimide, 5 mM magnesium sulphate), washed within 48 hours in transport medium lacking ammonium sulphate, and then frozen in chlordifluormethane R 22 at the temperature of liquid nitrogen.The specimens were stored at −70°C until processed. Cryostat sections, between 4 and 10 um thick were air-dried for 30 minutes before being incubated with primary antibodies.

Proteins and primary antisera

Monoclonal antibodies against fibrillin were produced by Sakai et al. (17). The working dilution was 1:200 (immunofluorescence) or 1:3000 (avidin biotin peroxidase complex technique).

FITC rabbit anti-human C3c, FITC rabbit anti-human IgA, FITC swine anti-rabbit IgG and TRITC goat anti-mouse IgG were from Dakopatts a/s Copenhagen. Polyclonal anti-IgA (working dilution 1:400) donated by DrBjörn Dahlbäck, Malmö. Biotinylated horse anti-mouse Ig were from Vector laboratories, Burlingame, USA. Monoclonal anti-vitronectin (working dilution 1:3000) was a kind gift from Dr Tamerius at Cytotech, San Diego.

Immunohistochemical techniques

To demonstrate colocalization of immunoreactivity of IgA with that of fibrillin in the dermal papillae, two staining procedures were used:

In *a double staining immunofluorescence procedure*, the specimens were first incubated with polyclonal anti-human IgA and then with FITC swine anti-rabbit IgG. Subsequently they were incubated first with anti-fibrillin and then with TRITC goat anti-mouse IgG after which the sections were examined in a fluroescence microscope with two filter systems, one selective for FITC, the other selective for TRITC. The distribution of anti- IgA and that of anti-fibrillin immunoreactivity were compared and photographs taken of all specimens.

In *a sequential procedure*, FITC rabbit anti-human IgA was used in the first reaction in the immunofluorescence technique. After photography, the sections were processed with monoclonal anti-fibrillin in an avidin-biotin-peroxidase complex technique, using biotinylated horse anti-mouse Ig as the secondary antiserum (21). Additional sections from three of the specimens (all of which had fibrillar IgA reactivity in the papillae) were stained by elastin stain in the second reaction. Photographs were taken of corresponding sections.

Negative controls using buffer, or unrelated antibodies instead of primary antibodies, were used in all experiments.

IgA and fibrillin reactivity was compared, using the sequential staining procedure on ten specimens and the double staining procedure on eight specimens. Both procedures were used on one of the specimens. Photographs of all sections stained by anti-IgA were compared with corresponding photographs of the same sections stained by anti-fibrillin (in either method) to ascertain whether the IgA reactive deposits were collocal with fibrillin reactive fibers.

RESULTS

Granules immunostainable by anti-IgA and anti-C3 were present in the tips of the dermal papillae in all the 17 specimens, in four of which there was additional fibrillar immunostaining by anti-IgA and anti-C3 in the dermal papillae. In all, but three specimens, fibrillin immunoreactive fibers extended perpendicularly towards the dermal epidermal junction in the papillary dermis, as in normal skin (18). In three specimens from elderly patients (72, 82 and 88 years old) fibrillin immunoreactivity was scarce in the upper part of dermis, except in a thin layer quite close to the junction, similar to previous findings in sunexposed skin from elderly people (18).

In specimens with granular IgA immunoreactivity, the IgA reactive structures were found collocal with fibrillin immunoreactive fibers in the papillary der-

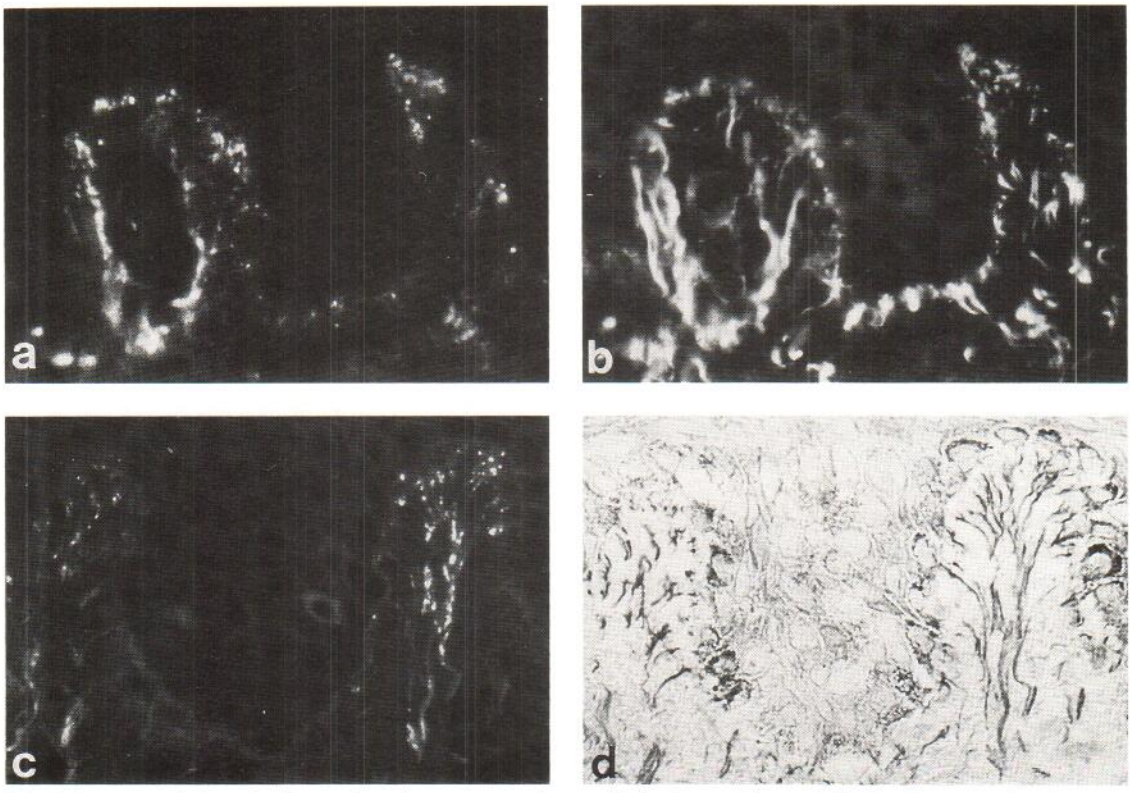

Fig 1. Immunostaining of skin sections from two patients with both granular and fibrillar IgA reactive deposits, demonstrating IgA immunoreactive deposits collocal with fibrillin immunoreactive structures in the dermal papillae. Sections of skin specimens stained with polyclonal anti-human IgA in an immunofluorescence technique (*a,c*), and with monoclonal anti-fibrillin in a double immunofluorescence technique using TRITC goat anti-mouse IgG as the secondary antibody (*b*), or in a sequential technique using biotinylated horse anti-mouse IgG as secondary antibody in an avidin-biotin peroxidase complex technique (d) (x 500).

mis in all cases, with the exception of a few IgA-reactive granules present at sites where there was faint or no fibrillin immunoreactivity. Owing to the limited resolution of the light microscopy technique the possibility could not be ruled out, that the presence of granules collocal with fibrillin reactive structures was merely a coincidence, at least in some of these specimens, especially in the case of specimens which had an abundance of fibrillin immunoreactive fibers in the papillary dermis and/or in which a large amount of granules were located in the immediate proximity of the junction.

However, it was easier to evaluate the four specimens whith the fibrillar IgA reactivity, in all four of which the IgA immunoreactivitity was clearly shown to be collocal with fibrillin immunoreactive fibers (Fig. 1). As discussed above, also in these specimens there were a few IgA reactive granules at sites lacking anti-fibrillin immunoreactivity.

Patterns of IgA immunoreactivity and those produced by elastin staining were compared in specimens from three patients with fibrillar type of IgA reactivity. Fibers in the middle and lower parts of the papillary dermis that were stained by IgA and anti-fibrillin were also found to be stainable by orcein (Fig. 2). The fibrillar IgA immunoreactivity extended further distally than the orcein-stained fibers.

DISCUSSION

This study was prompted by earlier reports on the ultrastructural association of IgA deposits with microfibrillar bundles in dermal papillae of DH patients, and the recent finding that fibrillin is a structural component of such microfibrils (11, 13–15, 17). The results confirmed those of earlier electron microscopy studies.

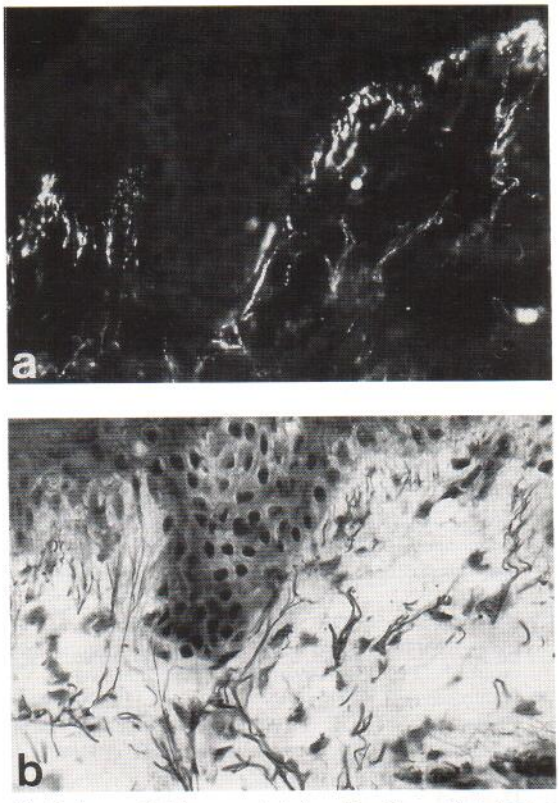

Fig 2. Sequential immunostaining of a skin section from a patient with fibrillar type of DH, demonstrating IgA immunoreactivity on orcein-stained fibers in the dermal papillae. A section of a skin specimen immunostained with FITC rabbit anti-human IgA in an immunofluorescence technique (*a*) and with elastin staining procedure (orcein) (*b*). (x 310)

In the specimens with fibrillar IgA reactive fibers, these were clearly collocal with the fibrillin immoreactive fibers, indicating that the IgA reactive deposits bind to fibrillin-containing microfibrils. Whether this also applies to all granular IgA deposits was not definitely established, and the possibility that granular IgA deposits may also bind to other structures could not be excluded.

The presence in some specimens of a few IgA-reactive granules at sites that were faintly or not at all stained by anti-fibrillin may indicate that some of the IgA-reactive granules lack association with fibrillin fibers. Alternatively, technical reasons may explain the absence of fibrillin immunoreactivity. For example, granules in DH skin are easier to stain and visualize with an immunofluorescence technique than with the avidin-biotin peroxidase complex technique when both techniques are optimized (unpublished observation).

The majority of the IgA reactive granules are located quite close to the dermal epidermal junction, a region where microfibrils and anchoring fibrils attach to the lamina densa (12). Immuno electron microscopicy findings have previously demonstrated the presence of IgA-reactive amorphous masses in conjunction with microfibrils in this region in skin from DH patients (11, 12–15). Further ultrastructural studies using anti-fibrillin and anti-IgA in immuno electron microcopical studies may provide further information, though the results may be difficult to interpret. Future immunochemical and biochemical studies on the nature of the IgA deposits are needed to elucidate what intermolecular mechanisms are involved. Hopefully, studies using purified components of these deposits and of extracellular matrix components, e.g. fibrillin, will be possible in the future.

The fibrillar IgA reactivity coincided with perpendicular orcein-stained fibers even though the fibrillar IgA reactivity extended further distally than the orcein-stained fibers in the dermal papillae. The perpendicular fibers have been termed "oxytalan" fibers. Like elastic fibers, they were recently found to have immunoreactivities of fibrillin, vitronectin and of serum amyloid P component and to be stainable by orcein in normal skin (18–20). However, in their distal parts which joined the dermal epidermal junction, they were stainable only by anti-fibrillin. This is the region where the majority of IgA reactive granules in DH skin are located. Thus, the IgA-reactive deposits in DH skin are most commonly located in a region where fibrillin normally lacks concomitant elastin and vitronectin. The finding that IgA-reactive granules are immunostainable by anti-vitronectin, suggests that vitronectin may be involved in the deposition of IgA-reactive material in skin of DH patients.

Hopefully, the hypothetical role(s) of fibrillin, vitronectin and other complement proteins in the formation of abnormal deposits in the skin of DH patients will be elucidated in future studies using biochemical and immunochemical techniques.

ACKNOWLEDGEMENTS

We thank all the physicians at the dermatology departments of the hospitals in Lund, Malmö, Kristianstad, Kalmar, Borås, Karlstad, Karlskrona,

Skövde, Sundsvall , Växjö and Boden, Sweden, who generously provided us with clinical data. We also thank Anne Ljungquist and Elise Nilsson for expert technical assistance. This work was supported by the Österlund foundation, the Welander foundation, the Finsen foundation and The Swedish Rheumatism Association.

REFERENCES

1. Immunoglobulins in the skin in dermatitis herpetiformis and their relevance in diagnosis. Br J Dermatol 1975; 92: 157–166.
2. Beutner EH, Chorzelski TP, Kumar V (eds) Dermatitis Herpetiformis. In: Immunopathology of the skin. 3rd edn., New York: John Wiley & Sons, 1987: 433–488.
3. Hall RP. The pathogenesis of dermatitis herpetiformis. Recent advances. J Am Acad Dermatol 1987; 16: 1129–1144.
4. Reunala T. Glutenfree diet in dermatitis herpetiformis. Br J Dermatol 1978; 98: 69–78.
5. Seah P, Fry L. Immunoglobulins in the skin in dermatitis herpetiformis and coeliac disease Lancet 1972; 18: 611–614.
6. Hall RP, Lawley. Characterization of circulating and cutaneous IgA immune complexes in patients with dermatitis herpetiformis. J Immunol 1985; 135: 1760–1765.
7. Karpati S, Török E, Kosnai I. Immunoglobulin A deposition in Jejunal Mucosa of children with dermatitis herpetiformis. J Invest Dermatol 1988; 91: 336–339.
8. Egelrud T, Bäck O. Dermatitis Herpetiformis: Biochemical properties of the granular deposits of IgA in papillary dermis. Characterization of SDS-soluble IgA-like material and potentially antigen-binding IgA fragments released by pepsin. J Invest Dermatol 1985; 84: 329–245.
9. Dahl MV, Falk RJ, Carpenter R, Michael A. Membrane attack complex in dermatitis herpetiformis. Arch Dermatol 1985; 121: 70–72.
10. Dahlbäck K, Löfberg H, Dahlbäck B. Vitronectin (S-protein) an inhibitor to the membrane attack complex of complement, colocalizes with Ig deposits and C9 neoantigen in discoid lupus erythematosus and dermatitis herpetiformis but not in bullous pemphigoid. Br J Dermatol 1989; 120: 725–733.
11. Yaoita H. Identification of IgA binding structures in skin of patients with dermatitis herpetiformis. J Invest Dermatol 1978; 71: 213–216.
12. Kobayasi T. Anchoring of basal lamina to elastic fibers by elastic fibrils. J Invest Dermatol 1977; 68: 389–390.
13. Guerra Rodrigo F, Cotta-Pererira G. Ultrastructural observations on uninvolved skin in dermatitis herpetiformis, elastic versus "reticular" fibrils. Br J Dermatol 1973; 89: 543.
14. Riches DJ, Martin BGH, Seah PP, Fry L. Ultrastructural observations on uninvolved skin in dermatitis herpetiformis. Br J Dermatol 1973; 88: 323–330.
15. Stingl G, Hönigsman H, Holubar K, Wolff K. Ultrastructural localization of immunoglobulins in skin of patients with dermatitis herpetiformis. J Invest Dermatol 1976; 67: 507–512.
16. Gibson MA, Kumaratilate J, Cleary EG. The protein components of the 12-nanometer microfibrils of elastic and nonelastic tissues. Biol Chem 1989; 264: 4590–4598.
17. Sakai LY, Keene DR, Engvall E. Fibrillin a new 350 kDa glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986; 103: 2499–2509.
18. Dahlbäck K, Ljungquist A, Löfberg H, Dahlbäck B, Engvall E, Sakay L. Fibrillin immunoreactive fibers constitute a unique network in the human dermis. J Invest Dermatol (in press).
19. Dahlbäck K, Löfberg H, Alumets J, Dahlbäck B. Immunohistochemical demonstration of age-related deposition of vitronectin (S-protein of complement) and terminal complement complexes on dermal elastic fibers. J Invest Dermatol 1989; 92: 727–733.
20. Breathnach SM, Melrose SM, Bhogal B, deBeer FC, Black M, Pepys MB. Immunohistochemical studies of amyloid P component distribution in normal human skin. J Invest Dermatol 1983; 80: 86–90.
21. Hsu SM, Raine L, Finger H. A comparative study of the peroxidase-anti-peroxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 1981; 75: 734–738.