IMMUNOELECTRONMICROSCOPIC LOCALIZATION OF IgA IN SKIN OF PATIENTS WITH DERMATITIS HERPETIFORMIS

HIDEO YAOITA, M.D., AND STEPHEN I. KATZ, M.D., PH.D.

Dermatologic Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, U. S. A.

Ultrastructural localization of in vivo-bound IgA in the skin of patients with dermatitis herpetiformis was determined by using a modified peroxidase–antiperoxidase multistep method. Three types of reaction product deposition are seen. The most common type of reaction product deposition, that which is identified by direct immunofluorescence as the speckled type of IgA deposit, shows up as clumps and yarnlike fibrils. The second type of IgA deposition, which is a linear band by direct immunofluorescence, appears to be associated with anchoring fibrils. The third type of IgA deposition, which is also linear by immunofluorescence, is confined to the lamina lucida.

Dermatitis herpetiformis (DH) is a chronic subepidermal blistering disease in which many immunologic abnormalities have been demonstrated [1, 2]. The most prominent of these is the almost universal presence of in vivo-bound IgA deposited in a granular or fibrillar pattern in the dermal papillae or in a continuous or linear pattern along the dermal–epidermal junction of normal and perilesional skin [3]. In this regard Seah and Fry [4] demonstrated in vivo-bound IgA in all 50 patients studied and we have made this observation in 38 of 40 patients diagnosed by clinical, histologic, and treatment response (to sulfones or sulfapyridine) criteria.

In order to determine the exact location of the IgA deposits in the skin of patients with DH and to gain insight into the structure(s) to which the IgA is bound, we have used an ultrastructural immunoperoxidase method [5]. In this paper we describe the ultrastructural localization of in vivo-bound IgA in the skin of patients with DH who have the granular or fibrillar type of deposits and in those who have the continuous of linear type of deposits.

MATERIALS AND METHODS

Patients. Each of the 6 patients studied had clinical and histologic evidence of DH and each responded dramatically to sulfones or sulfapyridine. There was a prompt recurrence of lesions when the medication was stopped. The normal and perivesicular lesional skin of each patient demonstrated in vivo-bound IgA in the dermal papillary tips (3 patients—2 men aged 33 and 73 and 1 woman aged 56) or at the dermal–epidermal junction (3 patients—2 women aged 42 and 55 and 1 man aged 33) using standard direct immunofluorescent techniques. No circulating antiepithelial antibodies were detected in the sera of any of these patients. Normal skin was also obtained from 2 volunteers.

Peroxidase–antiperoxidase (P-AP) multistep method. The tissue preparation and staining procedures were the same as described previously [5] except that a 1:40 dilution of fluoresceinated goat antihuman IgA (G/HA, Hyland; specific antibody concentration 2.2 mg/ml) was used for the demonstration of in vivo-bound Ig. Normal goat serum (NGS) was used as a control.

RESULTS

Light Microscopy

Speckled type. When the P-AP method was used, the dark brown clumps of reaction products were seen beneath the epidermis, especially in the dermal papillae of both normal and perilesional skin (Fig. 1). These deposits were the same shape, of equivalent size, and in the same distribution as those seen in the tissue stained by direct immunofluorescence (DIF) (Fig. 2). The epidermis and the rest of the dermis showed only very light yellowish brown staining which was considered to be background.

Linear type. The dark brown linear bands of reaction products were seen along the basement membrane zone (BMZ) of the epidermis in the normal-appearing and perilesional skin of 3
Fig. 1. DH skin with speckled-type IgA deposition stained by P-AP method. The dark brown clumps beneath the epidermis are the reaction product deposits which indicate IgA deposition (× 220).

Fig. 2. DH skin with speckled-type IgA deposition stained by immunofluorescent technique. The white clumps beneath the epidermis show the IgA deposits in the same pattern as in Fig. 1 (× 220).

Fig. 3. DH skin with linear (dermal)-type IgA deposition stained by P-AP method. The dark brown linear band along the epidermal-dermal junction shows the reaction products (× 220).

Fig. 4. DH skin with linear (dermal)-type IgA deposition stained by immunofluorescent technique. The white linear band along the epidermal-dermal junction shows IgA deposits in the same pattern as in Fig. 3 (× 220).

patients (Fig. 3). This pattern of IgA deposition was the same as that seen using DIF (Fig. 4). The other parts of the tissue showed the same light yellowish brown background as seen in the speckled type.

Electron Microscopy

There were no reaction products in the intercellular spaces and only sparse dark deposits in the deep dermis of all G/HA- and NGS-treated DH and normal human skin of the volunteers. G/HA-stained normal human skin showed no specific reaction products.

Granular (dermal) pattern. In the skin of patients with DH with the speckled pattern, all of the reaction products were seen in the dermis (Fig. 5). It was difficult to identify the components of the stained substances because they were usually very densely stained (Fig. 6). However, some of the stained structures had stringlike tails (80-130 Å diameter) and, in areas, looked like yarn (Fig. 7). It was not possible to determine whether the periodicity of the stringlike tails was regular since they were also stained very densely in most areas. Only a few of the specks in the upper dermis appeared to be associated with anchoring fibrils (Fig. 6). There was almost no staining of the elastic fibers.

Linear (dermal) pattern. In skin of 2 of the 3 patients with linear IgA staining by DIF, the reaction products were seen beneath the basal lamina in a nodular or reticular pattern (Fig. 8). These deposits were limited to the area where anchoring fibrils are usually seen. The periodicity of some of the anchoring fibrils was clearly accen-
Fig. 6. DH skin with speckled-type IgA deposition. The reaction products in the upper dermis appear to be associated with anchoring fibrils (circle) (× 48,000).

Fig. 7. DH skin with speckled-type IgA deposition. Dark granular-appearing clump which has strands (arrow) attached (× 51,200).

form only dark nodules or strings which were, at times, thicker than the anchoring fibrils and appeared reticular (Fig. 8).

Although almost all of the reaction products were seen in the dermis adjacent to the basal lamina, there were a few less-well-defined deposits somewhat deeper in the dermis, again where the basal lamina may have been present because of the tangential cut. In control sections treated with NGS, no specific reaction products were seen (Fig. 9).

Linear (lamina lucida) pattern. In this man's skin, the reaction products were deposited in the lamina lucida of the BMZ (Fig. 10). These deposits were seen mainly on the dermal side of the lamina lucida and there was a regularly well-demarcated space between the basal cell membrane and the reaction products, i.e., there was a well-defined less-stained space in the upper portion of the lamina lucida (Fig. 10). There were no specific reaction products seen below the basal lamina in contrast to the other 2 patterns where the staining was confined almost entirely to below the basal lamina. There were no reaction products in the epidermal cells except for accentuation of the basal cell membranes and half desmosome areas (Fig. 10). In this regard, this accentuation was also seen in control sections which were incubated with NGS instead of G/HA (Fig. 11).

DISCUSSION

In this study we demonstrated that in the skin of patients with DH there are three distinctly different patterns of IgA deposition when a P-AP method [5] is used for the ultrastructural localization of antibody. The granular or fibrillar deposits which are the most commonly seen in normal, appearing and perilesional skin of patients with DH are arranged in compact bundles and strands in the dermal papillary tips. The fibrillar configuration of these IgA deposits may resemble the structure of sub-basal lamina fibrous elements including microfibrillar bundles [6] and young collagen [7]. Exact identification is not possible as the procedure for this P-AP method differs from that of routine electron microscopy. The immunoperoxidase findings in 2 of our 3 patients who had linear IgA staining by immunofluorescent staining were similar to the IgG deposits demonstrated by Seah et al [8]. In these patients' skin, anchoring fibrils appear to be stained, in some places clearly, and in others the reaction products obscured their exact localization. The reaction products probably represent more than just antibodies to anchoring fibrils and may signify the binding of immune complexes.

The findings in the skin of the other patient with linear staining of IgA at the BMZ differ considerably in that the reaction products were found in the lamina lucida and there were no appreciable reaction products below the basal lamina. Suffice it to say that this patient met all of the accepted criteria for diagnosis as a DH patient [4]. A detailed description of this patient's clinical, histologic, immunologic, and ultrastructural findings are presented in detail in a succeeding paper [9]. We did not detect in vivo-bound IgG or circulating BMZ antibodies. The reaction products in this third type of IgA deposition were seen in a location similar though not identical to those immunoreactants deposited in the skin of patients with herpes gestationis (HG) [5] and bullous pemphigoid (BP) [10-12]. The difference between the location of these IgA deposits and those of HG and BP is that...
Fig. 8. DH skin with linear (dermal)-type IgA deposition. The dark granular-appearing reaction products (+) are seen in the dermis below the BMZ (B). The periodicity of some anchoring fibrils (square and inset) appears to be accentuated and that of others is obscured by the reaction products which appear to form reticular deposits. Sparse reaction products are seen in the dermis but no collagen is stained. E = epidermis; D = dermis (x 12,325; inset x 35,700).

Fig. 9. DH skin with linear (dermal)-type IgA deposition. Control for section stained in Fig. 8. This section was incubated with GSA instead of G/H. No reaction products are seen in the dermis. E = epidermis; D = dermis (x 20,800).

Fig. 10. DH skin with linear (lamina lucida)-type IgA deposition. The reaction products (+) are seen in lamina lucida. There is a fairly-well-defined electronlucent space (arrows) between the deposits and basal cell plasma membrane. No specific deposits of the reaction products are seen in the dermis. E = epidermis; D = dermis; BL = basal lamina; HD = half desmosomes; M = mitochondria (Counterstained with uranyl acetate and lead citrate, x 36,000).

Fig. 11. DH skin with linear (lamina lucida)-type IgA deposition. Control for section stained in Fig. 10. This section was incubated with NGS instead of G/H. No reaction products are seen in the lamina lucida. E = epidermis; D = dermis; LL = lamina lucida; BL = basal lamina; HD = half desmosomes (Counterstained with uranyl acetate and lead citrate, x 40,000).
in this patient's skin there is a well-defined space between the reaction products and the basal cell plasma membrane, whereas in HG the reaction products are seen throughout the lamina lucida and in BP a well-defined space is at times seen between the reaction products and the basal lamina. It is therefore suggested that there may be different antigenic structures in the lamina lucida. Jablonska et al [13] recently addressed themselves to the occasional difficulty in differentiating DH from BP. Immuno-electronmicroscopic identification of the localization of the in vivo-bound antibodies in the skin of their patients would undoubtedly help to further categorize the disease.

Several possibilities exist as to why the patterns of IgA deposition differ from one patient's skin to another. First, it may be that in the three types of DH skin there are different antigens or specific binding sites for IgA. Second, the skins of patients with DH may be antigenically similar to each other and there are different sites for the adherence of circulating immune complexes which deposit in various patterns. Both or other possibilities may be operating. Characterization of the antigen or of the specific IgA antibody will aid in understanding the importance and etiology of these IgA deposits.

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REFERENCES