Waldenström Macroglobulinemia–Induced Bullous Dermatosis

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Background: Waldenström macroglobulinemia is a plasma cell dyscrasia of undetermined cause characterized by the monoclonal proliferation of lymphoplasmacytes in the bone marrow, lymph nodes, and spleen and elevated circulating levels and tissue deposition of monoclonal IgM produced by these aberrant cells. Rarely, cutaneous manifestations of this disease have been reported.

Observations: We report the case of a patient with bullous dermatosis induced by Waldenstrom macroglobulinemia and demonstrate the subepidermal location of the separation and the presence of IgM and k light chains by immunoperoxidase, immunofluorescent techniques, and electron microscopy with immunogold staining. Immunoblotting revealed a strong band at the 290-kd area.

Conclusions: The demonstration of the separation in the upper dermis at the site of IgM deposits suggests that these deposits may be an etiologic factor in this rare manifestation.

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WALDENSTROM macroglobulinemia, a plasma cell dyscrasia of undetermined cause, is characterized by the monoclonal proliferation of lymphoplasmacytes in the bone marrow, lymph nodes, and spleen. Patients with this disorder typically have elevated circulating levels and tissue deposition of monoclonal IgM produced by these aberrant cells.1,2 This hematologic cancer is detected by a monoclonal IgM elevation on serum protein electrophoresis.3 The median age at the time of diagnosis is 63 years, and there is a slight predominance of the disorder in men. The most common presenting symptoms are weakness, fatigue, bleeding, weight loss, and visual or neurological disturbances.4 Other clinical manifestations caused by the elevated levels of circulating IgM include the hyperviscosity syndrome, cryoglobulinemia, and cold agglutinin–positive hemolytic anemia. Complications of the tissue deposition of IgM include neuropathy, glomerular disease, and amyloidosis.5

Cutaneous findings associated with Waldenstrom macroglobulinemia are rare but include urticaria, papular eruptions, nodules, ulcers, leukocytoclastic vasculitis, livedo reticularis, and flesh-colored translucent lesions containing deposits of IgM called “storage papules.”6-9 Vesiculobullous eruptions have also been reported10-13 in patients with Waldenstrom macroglobulinemia. The eruptions described have included the diagnoses of paraneoplastic pemphigus, bullous pemphigoid, and bullous drug eruption. We present what we think is the fourth reported case in the English-language literature of a patient with bullous dermatosis induced by Waldenstrom macroglobulinemia. We demonstrate the subepidermal location of the separation and the presence of IgM and k light chains by immunoperoxidase and immunofluorescent techniques as well as immunogold staining by electron microscopy. We further characterize the nature of the antibodies by demonstrating, via immunoblotting, that the patient has IgM antibodies to dermal extract protein with a band in the 290-kd area.

REPORT OF A CASE

A 41-year-old Hawaiian woman presented with a 2-month history of a tender, purple lesion on her left heel, intermittent purple discoloration of both feet that was exacerbated by prolonged standing, and the appearance of small blisters on her hands following minimal trauma. Her medical history was remarkable for periodic migraine

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METHODS

IMMUNOELECTRON MICROSCOPY

Immunelectron microscopy was performed using the saponin-permeabilization technique. Skin biopsy specimens were collected with a 6-mm punch and washed (nonfixed) in phosphate-buffered saline (PBS) blocking agent (1% bovine serum albumin, 1% normal goat serum, 0.1% fish gelatin, and 0.05% sodium azide at pH 7.4). Specimens were prefixed and prepared using a standard method previously described.14

Skin specimens were then incubated with anti–human light-chain (κ, Sigma Chemical Co, St Louis, Mo) monoclonal antibody at 1:25 or with purified mouse immunoglobulin (purified mouse IgG, Sigma Chemical Co) in washing buffer without saponin, which served as a control, at 4°C overnight. After 3 washes, the skin specimens were incubated with the Fab’ portion of a goat anti–mouse IgG antibody conjugated with colloidal gold particles (1.4-nm gold particle, Nanoprobe Inc, Stony Brook, NY) at 4°C overnight. Skin specimens were then washed in PBS and postfixed in half-strength Karnovsky fixative. Silver enhancement was performed according to the method described by the manufacturer (HQ Silver Enhancement Kit, Nanoprobe Inc). After being washed with a solution of 0.1-mol/L PBS and then in distilled water, the specimens were osmicated in 1% osmium tetroxide in a solution of 0.1-mol/L PBS for 30 minutes at room temperature. Specimens were prepared for ultrathin sectioning by standard embedding procedures. Finally, ultrathin sections were placed on copper-mesh grids and poststained with uranyl acetate and lead citrate lead for contrast.

IMMUNOBLOTTING WITH HUMAN DERMAL EXTRACT

Procedures used to evaluate serum specimens for human dermal skin blots vs alkaline phosphate goat anti–human IgM antibodies involved performing polyacrylamide gel electrophoresis using 8% sodium dodecyl sulfate with a 4% stacking gel in a discontinuous system (Laemmli-Ornstein-Davis).13 The human dermal extract was prepared using the method delineated by Zhu et al.14 Then, a 320-µL specimen was loaded into the large well, and 10 µL of a standard control mix was loaded into the small well. The sample buffer contained the reducing agent dithiothreitol (Sigma Chemical Co).

Electrophoresis was performed. The gel was then washed in destain for 10 minutes. The washed gel was then loaded to a blotting apparatus (Genie, Ideal Scientific, Corvallis, Ore) and was blotted to nitrocellulose (Schleicher & Schuell Inc, Keene, NH) at room temperature for 60 minutes. The section of nitrocellulose containing the standards and part of the human protein extract for India ink staining was excised and left at room temperature overnight. The remainder of the nitrocellulose was blocked with a solution of 0.05-mol/L Tris buffer, pH 8.0, and 1% nonfat dried milk at 37°C for 30 minutes. The nitrocellulose was then washed and stored until the immunoblot procedure was done.

Immunoblotting was accomplished by placing nitrocellulose strips in lanes of an Accutan tray (Schleicher & Schuell Inc), adding 250 µL of 1:30 dilute sample (diluent is 0.9% sodium chloride to 0.1% polysorbate 20) to each lane, incubating at room temperature overnight, and then washing with the sodium chloride–polysorbate 20 solution. Then 250 µL of 1:500 diluted alkaline phosphate–labeled goat anti–human IgM (Sigma Chemical Co; catalog No. A3275). Enzyme conjugate diluent was a solution of 0.1-mol/L PBS, pH 7.2, 0.1% polysorbate 20, and 2% normal rabbit serum. This was incubated at room temperature for 2½ hours. Next, 300 µL of a combination of BCIP and nitroblue tetrazolium substrate (Sigma Immunochromics; catalog Nos. B9503 and N6876) was added to each lane and incubated at room temperature for 30 minutes. The substrate reaction was stopped by washing the nitrocellulose strips 4 times with distilled water.

headaches for the past 6 years and iron-deficiency anemia. Her family history was noncontributory.

On physical examination, she appeared healthy, with relevant findings limited to her distal extremities. On the dorsal surfaces of both hands, she had several 2- to 4-mm hypopigmented scars and an excoriating. No acute lesions or vesicles were initially noted. Both feet were cool to palpation, with extensive nonpalpable, blanchable purpura that became more pronounced when the feet were placed in a dependent position. Her left heel demonstrated an exquisitely tender, 2-cm purple plaque with overlying scale and minimal induration.

Relevant laboratory data included a white blood cell count of 7.3 × 10^9/L, with 0.49 lymphocytes (reference range, 0.18-0.44), 0.17 of which were atypical, and a Westergren erythrocyte sedimentation rate of 61 mm/h (reference range, 0-20 mm/h). The results of tests for hematocrit, hemoglobin level, liver function studies, antinuclear antibody profile, prothrombin and partial thromboplastin times, perinuclear–antineutrophil cytoplasmic autoantibody and cytoplasmic–antineutrophil cytoplasmic antibody levels, cryoglobulins, cryofibrino-gens, serum viscosity, and urine porphyrin screen were normal or negative. Complement levels were decreased, with a CH50 of 165 (reference range, 189-420). Serum protein electrophoresis revealed an elevated IgM level of 22.8 g/L (reference range, 0.6-2.6 g/L) and a κ light-chain level of 14.6 g/L (reference range, 5.7-12.8 g/L). Levels of IgA and IgG, γ-globulin, and λ light chain were within the reference ranges. The κ-to-λ light-chain ratio was 3.31 (reference range, 1.35-2.69).

Left upper quadrant sonography delineated an enlarged spleen. The patient was referred to the hematology-oncology service for further evaluation and diagnostic procedures. A bone marrow biopsy specimen showed an increased number of abnormal plasma cytoid cells, and the diagnosis of Waldenstrom macroglobulinemia was made.

Before the results of the serum electrophoresis were obtained, an excisional biopsy was performed on a purpuric area on the left foot away from the symptomatic lesion to exclude vasculitis as a possible diagnosis. Routine staining with hematoxylin and eosin revealed small and large vessels of the dermis and subcutis to be oc-
cluded by eosinophilic material (Figure 1). Significant inflammation was absent. This material stained strongly with periodic acid–Schiff after digestion with diastase but did not stain with phosphotungstic acid hematoxylin. This staining pattern is consistent with deposited immunoglobulin and excluded fibrin clots as the cause of the occluded vessels. Immunoperoxidase studies demonstrated the amorphous material to be decorated with \( k \) light-chain but not \( \lambda \) light-chain antibodies. At this time, it was also noted that the basement membrane zone demonstrated linear \( k \) light-chain staining (Figure 2).

Prompted by a finding on gynecological examination, imaging of the pelvis revealed a right adnexal mass. Exploratory laparotomy with hysterosalpingo-oophorectomy was performed. The uterus and cervix demonstrated occlusion of vascular lumina by amorphous material similar to that seen in the skin biopsy specimen. During that hospital stay, the patient had a vesicle develop on the dorsum of her right hand. Histological examination of this lesion displayed subepidermal separation on routine preparation. Part of this lesion was examined using the direct immunofluorescent technique and showed dermal deposition of IgM (Figure 3). Indirect immunofluorescence using normal human skin test substrate demonstrated that the plasma specimen contained an IgM anti–epidermal basement membrane zone antibody. Electron-microscopic evaluation demonstrated amorphous deposits in the superficial dermis just below the lamina densa, with the separation in the superficial dermis occurring in the area of the amorphous deposits (Figure 4). Identical amorphous material was present in skin that did not exhibit a separation. Immunogold staining with anti–human IgM established that the amorphous material seen on electron microscopy was indeed immunoglobulin (Figure 5).

Plasmapheresis was performed because of concern that the vessel occlusion seen in the skin and internal organs might be occurring in the central nervous system. Immunoblotting using the patient’s serum and human dermal extract revealed a strong band at the 290-kd area. Control patients’ serum demonstrated no band (Figure 6).

**COMMENT**

We describe a case of bullous dermatosis in a patient with Waldenström macroglobulinemia and demonstrate that the location of the separation of the vesicle was subepidermal with dermal deposits of IgM \( k \) light-chain isotype. The location of the IgM deposits suggests that they may be responsible for the blister formation. The electron microscopic localization of splitting in the dermis at the site of the IgM deposits suggests this as a possible etiologic factor. Other indications include the binding of IgM to normal skin and binding to a 290-kd protein, presumably anchoring fibrils in the dermis.

The mechanism by which the deposition of immunoglobulins causes vesicular formation is unknown; however, the concept of immunoglobulin being deposited much more extensively than in the sites of blistering is not novel. The classic example is pemphigus vulgaris, where antibodies are known to be pathogenic and are diffusely deposited, but blisters are usually localized. Wuepper et al described the case of a patient with Waldenström macroglobulinemia and a concomitant bullous eruption whose macroglobulin in situ bound anti-
epidermal basement membrane zone antibody, and circulating anti–epidermal basement membrane zone antibodies were of the IgM k light-chain isotype. Similarly, Whittaker et al\(^{17}\) described a case of a vesicular eruption in a patient with Waldenström macroglobulinemia in which they established that IgM deposits were located in the roof and base of a specimen of 1-mol/L sodium chloride–split skin from the patient. On indirect immunofluorescence, the patient's serum had circulating antibodies that bound to the roof and the base of sodium chloride–split skin substrate. The Table summarizes the cases of bullous eruptions reported in patients with Waldenström macroglobulinemia and the observed immunofluorescence patterns. In addition, Cobb et al\(^{18}\) described the case of a patient with Waldenström macroglobulinemia who had a distinctive papular eruption and immunopathological findings, suggesting that this paraprotein had specificity for the epidermal basement membrane zone. This was demonstrated by the binding of the patient’s circulating IgM anti–epidermal basement membrane zone antibody to both sides of 1-mol/L sodium chloride–split skin (dermal side greater than epidermal side). A lamina lucida epitope was the implied target for the antibody based on split-skin indirect immunofluorescence studies.

A proposed mechanism of blister formation in our patient may be similar to that seen in patients with epidermolysis bullosa acquisita or bullous systemic lupus erythematosus. The epidermolysis bullosa antigen is the globular C-terminal domain of type VII procollagen. Antibody reacts with both a 145-kd and a 290-kd protein. Type VII collagen is the major structural component of anchoring fibrils that link the lamina densa above to the dermis below. In bullous systemic lupus erythematosus, the antibodies associated with the subepidermal immunoglobulin deposits bind beneath the lamina densa and react with the same antigen involved in epidermolysis bullosa acquisita, type VII procollagen.\(^{18}\) Our patient had neither of these diseases. The 290-kd protein antibody identified in our patient’s serum may also bind to the anchoring fibrils and interfere with their assembly in a manner similar to that seen in bullous systemic lupus erythematosus and epidermolysis bullosa acquisita. The presence of antibody along the dermoepidermal junction, as demonstrated by immunofluorescence and immunogold electron microscopy in this patient’s skin, supports this. To our knowledge, this is the first case of a bullous eruption in Waldenström macroglobulinemia studied with electron microscopy and the immunogold technique, although Lipsker et al\(^{19}\) used this method to study storage papules of this condition.
Cases of Waldenström Macroglobulinemia-Induced Bullous Dermatoses

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Clinical Finding(s)</th>
<th>Histological Features</th>
<th>Direct Immunofluorescence</th>
<th>Indirect Immunofluorescence</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/70</td>
<td>Chronic bullous eruption for 4 y</td>
<td>Vesicles at dermal-epidermal junction</td>
<td>IgM-k, C3 at dermal-epidermal junction</td>
<td>IgM-k anti–basement membrane zone antibody</td>
<td>Wuepper et al,10 1978</td>
</tr>
<tr>
<td>2/F/83</td>
<td>Diffuse bullae for several months</td>
<td>Subepidermal separation</td>
<td>Linear IgM on basement membrane of normal and lesional skin</td>
<td>IgM anti–basement membrane zone antibody</td>
<td>Got pel et al,11 1982</td>
</tr>
<tr>
<td>3/M/62</td>
<td>Vesicles on forearms and trunk</td>
<td>Subepidermal separation</td>
<td>Linear IgM on basement membrane of normal and lesional skin</td>
<td>IgM anti–basement membrane zone antibody</td>
<td>Whittaker et al,12 1996</td>
</tr>
<tr>
<td>4/F/41</td>
<td>Intermittent vesicles on hands</td>
<td>Subepidermal separation</td>
<td>Dermal deposition of IgM in normal and lesional skin</td>
<td>Anti-IgM antibody</td>
<td>Present study</td>
</tr>
</tbody>
</table>

CONCLUSIONS

In addition to demonstrating a subepidermal location of the separation of bullous dermatosis in a patient with Waldenstrom macro- globulinemia, this case also demonstrates the ability to use immunoperoxidase to show IgM k light chain in the basement membrane zone and in blood vessels. Studies using indirect immunofluorescence, electron microscopy, and immunogold staining documented the deposition of circulating IgM in the superficial dermis, with blister formation in the area of the deposits. The investigation of additional patients with similar clinical features may help to determine whether our patient’s antibodies were unique in their specificity for skin.

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REFERENCES


