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IMMUNOREACTIVITY TO DERMAL VESSELS IN A PATIENT WITH PYODERMA GANDRENOSUM

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Abstract

Introduction: Pyoderma gangrenosum (PG) represents a lesion with an elusive etiology associated with Crohn's and/or arthritic diseases. A genuine associated vasculitis has not been proven; however, dilation of the dermal blood vessels the presence of neutrophilic and/or lymphocytic infiltrates in selected biopsies suggests that vessels are likely play a role in the pathogenesis of PG.
Materials and Methods: A patient presented with a necrotic pustule or furuncle, evolving into a large necrotic ulcer with violaceous borders and surrounding erythema. A skin biopsy for hematoxylin and eosin (H&E) review and immunohistochemistry (IHC) stains was obtained. A second biopsy for direct immunofluorescence (DIF) was also taken.
Results: H&E review demonstrated an ulcerated epidermis; within the ulcer base were numerous neutrophils, lymphocytes, histiocytes and fibrin. No vasculitis was present. DIF revealed strong deposits of FITC conjugated fibrinogen around superficial and the deep dermal vessels. FITC conjugated Complement/C1q and albumin conjugated were also seen between dermal extracellular matrix fibers. IHC showed that the dermal vessels (venules, arterioles and lymphatics) displayed dilation, and a loss of normal endothelial markers including von Willebrand factor and D2-40/podoplanin.
Conclusions: Our case of PG shows that there seems to be an alteration of several skin structures, including dermal vessels. An alteration of the vascular fibrin-fibrinogen balance was also detected, causing some autoreactivity to fibrinogen and an immune response involving neutrophils and T lymphocytes. Our findings suggest that the etiology of PG is more complex than previously thought.

Key words: Pyoderma gangrenosum; von Willebrand factor; vascular degeneration, autoimmunity; lymphatic vessels; CD99; D2-40

Abbreviations and acronyms: Pyoderma gangrenosum (PG), immunohistochemistry (IHC), direct immunofluorescence (DIF), hematoxylin and eosin (H&E).

Introduction

Pyoderma gangrenosum (PG) is an uncommon cause of painful skin ulceration. It may affect any part of the skin; however, it mainly presents on the lower legs, and has an unknown etiology resembling a Class II Schwartzman-like hypersensitivity reaction. PGs usually initially present as a single pustule, progress to solitary or multiple erythematous macules and papules and then to ulcerated, painful nodules or plaques with undermined, dusky borders. Patients are often systemically ill, with associated symptoms such as fever, malaise, arthralgias, and myalgias. Lesional pain may be severe. When lesions heal, the scars are often cribriform. PG is sometimes classified as a neutrophilic dermatosis, and has often been considered a reaction to another internal disease such as Crohn's disease, ulcerative colitis, polyarteritis nodosa or hematologic alterations. PG may affect males and females of any age, but is more common in people over 50 years [1-5]. PG combined with acne and a pyogenic arthritis may represent a rare genetic disorder characterized by its effects on skin and joints (PAPA syndrome).

Case Report

A 58 year old female presented to her dermatologist for evaluation of a pustule or furuncle that was evolving into an ulcer. The patient denied any gastrointestinal, acne or joint problems concomitant with the presentation of the skin lesions. Physical exam revealed a large, necrotic ulcer with violaceous border and surrounding erythema.

On examination, the ulcer was tender. Skin biopsies were obtained for hematoxylin and eosin (H&E) review, immunohistochemistry (IHC) staining and direct immunofluorescence (DIF) studies.

Material and Methods

For DIF, we incubated 4 micron glass slides with our secondary antibodies as previously described [6-9]. We utilized FITC conjugated rabbit anti-total IgG (Dako, Carpinteria, California, USA) at a 1:25 dilution. The samples were run with positive and negative controls. We also utilized FITC conjugated rabbit antisera to human IgG, IgA, IgM, Complement/C1q, Complement/C3, fibrinogen and albumin. Anti-human IgA antiserum (alpha chain) and anti-human IgM antiserum (mu chain) were obtained from Dako. Anti-human IgE antiserum (epsilon chain) was obtained from Vector Laboratories (Burlingame, California, USA). Anti-human IgD FITC-conjugated antibodies were obtained from Southern Biotechnology (Birmingham, Alabama, USA). The slides were counterstained with 4',6-diamidino-2-phenylindole(DAPI) (Pierce, Rockford, Illinois, USA). A mouse anti-collagen IV monoclonal antibody (Invitrogen, Carlsbad, California, USA) was also utilized, with secondary donkey anti-mouse IgG antisera (heavy and light chains) conjugated with Alexa Fluor 555 (Invitrogen).

Immunohistochemistry (IHC)

We performed IHC utilizing multiple monoclonal and polyclonal antibodies, all from Dako (Carpinteria, California, USA). For all our IHC testing, we used a dual endogenous peroxidase blockage, with the addition of an Envision dual link (to assist in chromogen attachment). We applied the chromogen 3,3-diaminobenzidine(DAB), and counterstained with hematoxylin. The samples were run in a Dako Autostainer Universal Staining System. Positive and negative controls were consistently performed. Our studies were specifically performed as previously described [6-9].The following antibodies were utilized: 1) monoclonal mouse anti-human CD99, MIC2 gene products, Ewing's sarcoma marker Clone 12E7, monoclonal mouse anti-human, 2) von Willebrand Factor, Clone F8/86, 3) polyclonal rabbit anti-human myeloperoxidase, monoclonal mouse anti-human neutrophil elastase Clone NP57, 4) polyclonal rabbit anti-human somatostatin, 5) monoclonal mouse anti-human podoplanin, Clone D2-40, 6) monoclonal mouse anti-human CD4, Clone 4B12, 7) monoclonal mouse anti-human CD45, Leucocyte Common Antigen Clones 2B11 + PD7/26, 8) monoclonal mouse anti-human CD8, Clone C8/144B, 9) monoclonal mouse anti-human IMP3 (Clone 69.1), (insulin-like growth factor II mRNA binding protein 3, 10) monoclonal mouse anti-human CD68 Clone EBM11, 11) monoclonal mouse anti-metallothionein Clone E9, 12) monoclonal mouse anti-human tissue inhibitor of metalloproteinases 1 and 13) polyclonal rabbit anti-human alpha-1-antitrypsin.

Resulrs

H&E tissue sections demonstrate an ulcerated epidermis (Fig. 1). Within the ulcer base were numerous neutrophils, lymphocytes and histiocytes; abundant fibrin was also present. Several vessels were dilated (Fig. 3). No vasculitis was noted; a dermal perivascular and interstitia infiltrate of lymphocytes and mononuclear cells was present. Fungal and bacterial stains were negative for organisms.

Direct immunofluorescence (DIF)

DIF displayed the following results: IgG (+, focal deep dermal perivascular); IgG3(-); IgA (++, focal deep dermal perivascular); IgM (+); IgD (-); IgE (-); Complement/C1q (++, focal deep dermal perivascular); complement/C3 (+, focal deep dermal perivascular); complement/C4(-); albumin (++, superficial dermal perivascular) and fibrinogen (++++, superficial and deep dermal perivascular, and around eccrine gland supply vessels). Fibrinogen was the strongest immunoreactant seen around the upper and deep dermal vessels, with Complement/C1q of second strongest intensity (Figs 1 - 4). In Figure 4, using Alexa 555 conjugated anti-human IgG antibody (red staining) we show positive staining of possible damaged vessels, amalgamated with some dermal structures

IHC staining

CD99 staining was very positive on dermal vessels subjacent to and surrounding the ulcer (Fig. 2). Somatostatin was weakly positive, within the dermal extracellular matrix. Our most important finding was the presence of significantly dilated lymphatic vessels via D2-40 staining, especially surrounding and subjacent to the ulcer (Fig. 2) Myeloperoxidase staining was positive, and showed some cellular defragmentation around one edge of the ulcer. Neutrophil elastase, CD4 and CD8 staining were predominantly negative. CD45 staining was positive in only few focal perivascular areas, adjacent to the ulcer. Anti-insulin-like growth factor II/mRNA binding protein 3 staining was negative. Of note, many dermal vessels were involuted and significantly damaged, observed by staining alterations with the von Willebrand antibody (Fig. 3).

Discussion

PG onsets are rapid, often at the site of a minor injury. The lesion may begin as a small pustule, a red bump or a blood-blister, with these lesions subsequently progressing to an ulcer. The ulcer often grows rapidly; the edges are usually purple and very painful and tender. Multiple ulcers may progress at the same time [1-5]. An ulcer may spontaneously improve, and complete healing may take months. It is important to evaluate if concomitant local venous disease is present, because this may play a negative role in the healing process. The clinical pathergy test is frequently positive (a skin prick test causing a papule, pustule or ulcer). Peristomal PG occurs close to abdominal stomas, and comprises about 15% of all cases of PG [1-2]. Most of these patients have inflammatory bowel disease, but peristomal PG may also occur in patients who have had an ileostomy or colostomy for either malignancy or diverticular disease. However, about 50% of those affected by PG demonstrate none of these associated risk factors.

Clinical differential diagnoses of PG lesions include antiphospholipid antibody syndrome, anthrax, Wegener's granulomatosis, atypical mycobacterial infections, Sweet's syndrome, venous or arterial disease, and Behçet's disease [1-5]. A final diagnosis of PG is based on the clinical appearance, and on ruling out histopathologic features of other disorders. There is no specific positive laboratory test for PG. However, it is recommended to swab PG ulcers for superinfecting pathogens. In our case, these studies were negative. It is also recommended to perform a chest radiogram. Angiography
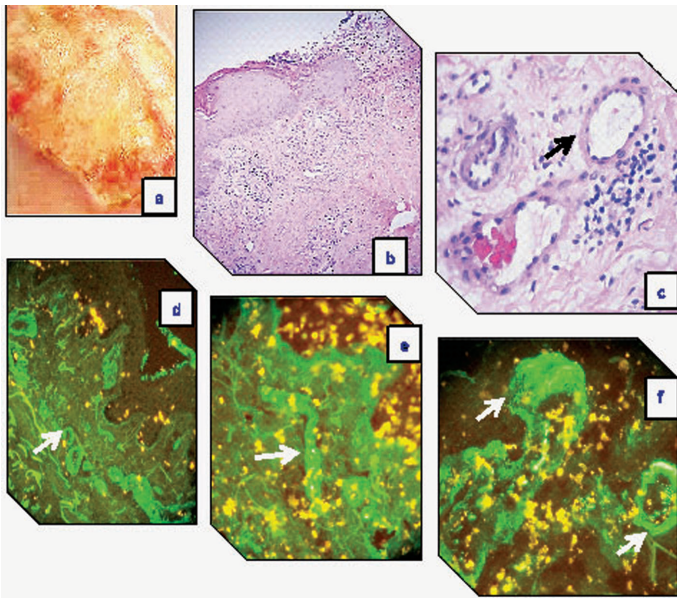
Figure 1. a, Clinical PG photograph, showing the PG ulcer. **b,c.** H&E photos at lower and higher magnification, respectively. In **b,** an ulcerated epidermis with numerous neutrophils, lymphocytes and histiocytes within the ulcer base; fibrin was also present. In **c,** we show several dilated dermal blood vessels. **d-f.** DIF. **d-f.** IgG conjugated with Alexa 555 (yellow staining) and FITC conjugated anti-fibrinogen(white arrows; green staining) on dermal lymphatic vessels.
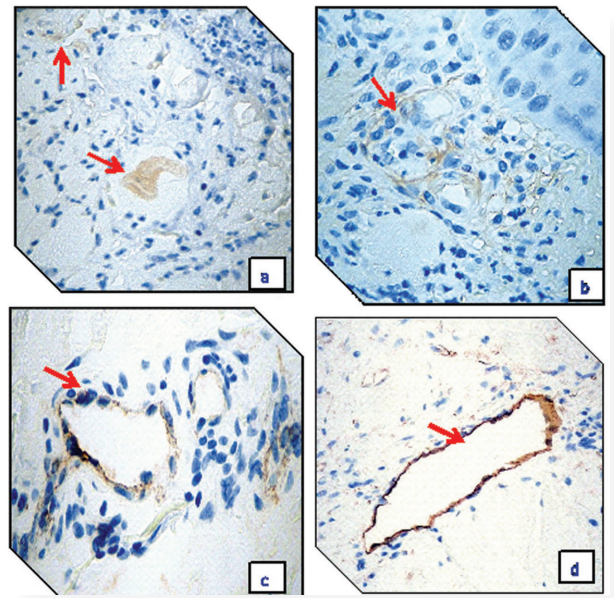

Figure 3. a-c. IHC staining, demonstrating damage to dermal vessels (showing degenerative changes and uneven staining with von Willebrand antibody (red arrows; light brown staining). **d.** Positive IHC staining, demonstrating a large, dilated lymphatic vessel via D2-40 antibody (red arrow; brown staining).
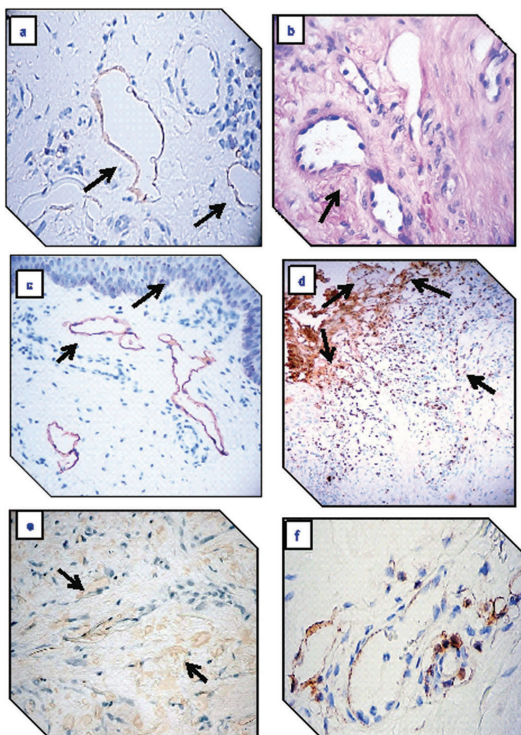

Figure 2. a, c, and **f,** CD99 positive IHC staining on dermal vessel endothelia(brown staining, black arrows). **b.** H & E staining, demonstrating dilated vessels in the dermis (400x) **d.** Positive IHC staining for myeloperoxidase (black arrows; brown staining). **e.** Positive IHC somatostatin staining in small dermal vessels of unknown identity (black arrows; light brown staining color).
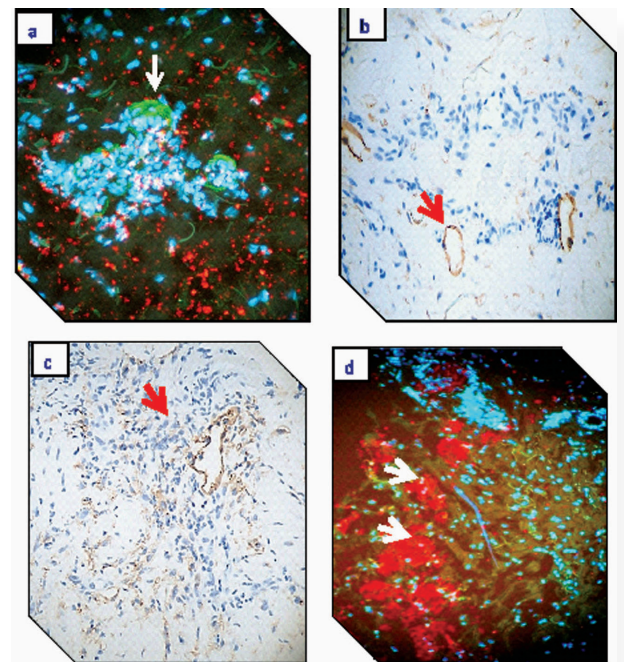

Figure 4. a. DIF, with Alexa 555 conjugated IgG (red staining) shows a tremendous deposition on some dermal structures (scattered red dots), and FITC conjugated fibrinogen (white arrow; green staining). Cell nuclei were counterstained with DAPI (blue). **b.** Positive D2-40 IHC staining documenting dilated dermal lymphatics (red arrow; brown staining) and in **c,** evidence of lymphatic vessel endothelial fragmentation(red arrow; brown staining). **d.** Positive DIF staining with Alexa 555 conjugated IgG (red staining) showing possible damaged vessels amalgamated with unidentified dermal structures (white arrows). Cell nuclei were counterstained with DAPI (blue).

Angiography or Doppler studies may be carried out in patients suspected of having arterial or venous insufficiency, and colonoscopy or other testing may be warranted to exclude associated inflammatory bowel disease in selected patients [1-5].

PG treatment includes the gentle removal of necrotic tissue. Small ulcers may be treated with potent topical steroid creams, intralesional steroid injections, and special dressings including silver sulfadiazine cream, potassium iodide or hydrocolloids. In some instances, oral anti-inflammatory agents such as dapsone or minocycline may help. If tolerated, careful compression bandaging may be helpful for swollen legs. Some severe cases warrant extended, systemic immunosuppressive therapy. The focus of treatment in these patients is long-term immunosuppression, often with high doses of corticosteroids or low doses of cyclosporin [2,3]. Lately, good outcomes have been reported for treatments with anti-tumor necrosis factor α; infliximab has also proven effective in a randomized, controlled clinical trial.

The etiopathology of PG is not clear. Some people believe that neutrophils play a critical role in this disease, because is common to see a neutrophilic dermal inflammatory infiltrate (as we found); however, this finding is not always present. Some patients may have positive ANCAs (antineutrophil cytoplasmic antibodies). In our case, these antibodies were negative. We found many defragmented neutrophils via positive staining for myeloperoxidase at the edges of the ulcer. Most T lymphocytic stains were negative (ie, CD4 and CD8; a few CD45 positive cells were noted). We found altered dermal vessels, and some degeneration of the vessels as demonstrated by altered staining of von Willebrand factor (especially under the ulcer and in close proximity). We also found many dermal vessels in these areas to be dilated, including the lymphatics; and we detected a immune response to these vessels, as demonstrated by positive CD99 staining in many of them. In addition, strong staining with anti-fibrinogen against these vessels was clearly present. The specific etiologic role of this vascular immune response is not known.

Other authors had reported clinical, histologic, and immunofluorescent findings in 68 PG cases. Notably, 78 per cent had associated systemic diseases, with arthritis and inflammatory bowel disease being most common. These authors reported cutaneous histopathologic changes varied with the site of biopsy. Specifically, lymphocytic vasculitis was predominant in the zone of erythema peripheral to the ulcers, while neutrophilic infiltrates and abscess formation were more prominent in central ulcer areas. In most of the cases studied, DIF showed immunoglobulins and complement deposited in and around superficial and deep dermal vessels, as we also observed [10].

Other authors reported the results of biopsies from ulcer edges in 8 PG patients. These biopsies were examined by DIF. Deposits of Complement/C3 were seen in the vessel walls of all samples, and IgM in three and IgA in one. Granular deposits of Complement/C3 were seen at the dermal-epidermal junction in 2 patients. Biopsies from clinically normal skin of 6 of the patients were DIF negative [11]. In agreement with our findings, these authors suggested that deposition of immune complexes in multiple dermal vessel walls may play a role in the pathogenesis of PG [11].

Conclusion

PG presents with multiple clinical appearances, and is easy to misdiagnose. The histopathology of PG is nonspecific; a biopsy is needed to exclude other diagnoses. Given our data, multiple dermal vessels including lymphatics, venules and arterioles seem to play a role in the immunopathology of PG.

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