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Disseminated Pyoderma Gangrenosum: Role for Vascular Endothelial Growth Factor and Hypoxia Inducible Factor-2

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Pyoderma gangrenosum; disseminated; visceral; spleen; lung; pleural effusion; empyema; ulcerative colitis; steroid; cyclosporine

To the editor

Pyoderma gangrenosum (PG) is a common manifestation of inflammatory and neoplastic disorders. Clinically, these lesions appear as deep ulcers that are extremely slow to heal. It may occur cutaneously as well as viscerally ¹. These lesions may be due to defects that prevent normal resolution of inflammation. No universally effective therapy exists for PG.

The index patient had an unusually severe case of PG, involving viscera as well as skin. In order to gain insight into the signaling processes underlying PG, we analyzed the production of vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2), as well as a transcription factor upstream of these molecules, hypoxia inducible factor 2- α (HIF2 α). We then stained additional samples from 3 additional patients to determine whether our findings can be generalized to PG. High level expression of VEGF and HIF2 α was observed in all lesions. Ang-2 was focally positive in PG lesions

Formalin-fixed paraffin embedded sections from punch biopsies of PG lesions at 5 microns from four cutaneous PG cases were stained as below. Antigen retrieval was performed using target retrieval solution at pH 6.0, diluted 1:10. HIF2 α , angiopoietin-2, and VEGF. The following primary and secondary antibodies and dilutions were used: Rabbit Polyclonal antibody to HIF2 (Novus Biologicals, Littleton, CO) catalog # NB100-122. Concentration 1:400; Rabbit Polyclonal anti-VEGF antibody (Santa Cruz Biolabs), Concentration 1:20;

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Anti-angiopoietin-2 Goat polyclonal antibody (Santa Cruz Biolabs, goat polyclonal), concentration 1:25. Strong nuclear staining for HIF2a and cytoplasmic staining for VEGF was observed in all lesions with staining of both neutrophils and neovasculature observed. Focal staining for angiopoietin-2 was observed in the lesions, but not as prominently as VEGF or HIF2a (Figure 1).

While the etiology of PG is unknown, the epidemiology of PG implies a defect in neutrophil function. Once the inflammatory stimulus is eliminated, the oxidative burst shuts off, the neutrophils die and inflammation ceases. In PG, there is no deficit in neutrophil recruitment and chemotaxis to a site of inflammation. The prolonged course of the disease suggests defects in the shutdown of the oxidative burst and in the resolution of neutrophilia. Sustained reactive oxygen lesions have been shown to induce angiogenesis, in part through the reactive oxygen sensor, hypoxia inducible factor-2 (HIF2) which induces angiogenesis by stimulating production of vascular endothelial growth factor (VEGF) and angiopoietin-24. We demonstrate (Figure 1) that the lesions of PG produce high levels of HIF2a and its downstream targets, thus implicating that PG may be a disorder of aberrant reactive oxygen production and may be angiogenesis dependent (Figure 2).

Our findings suggest at least two novel targets for the treatment of PG. First, VEGF (produced abundantly by PG lesions) may be blocked with drugs such as bevacizumab (Avastin). Second, drugs that target HIF2/reactive oxygen signaling pathways may be of benefit. We have previously shown that triphenylmethane dyes decrease the production of angiopoietin-2 (a downstream effector of HIF2) in endothelial cells through inhibition of NADPH oxidases (nox enzymes). One such compound, gentian violet, is FDA approved and is a potent inhibitor of nox2/HIF2 signaling 4. While gentian violet was not used in these patients, a clinical trial of a foam containing gentian violet was found to be efficacious in several cases of PG 5. Our findings suggest at least two steps of a pathway in which angiogenesis may be blocked and may enhance the treatment of PG without extensive immunosuppressive measures.

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Figure 1.

Figure 1 series. First row (1A, 1B); Second row (1C, 1D); Third row (1E, 1F).

Figure 1A. Representative skin lesion of patient upon admission, demonstrating deep ulceration and heaped up edges.

Figure 1B. Computed tomography scan of the patient revealing splenic hypodensities.

Figure 1C. Histology of patients skin lesion, representing hematoxylin and eosin staining of the skin lesion.

Figure 1D. represents immunohistochemistry for angiopoietin-2.

Figure 1E. represents immunohistochemistry for VEGF.

Figure 1F. represents immunohistochemistry for HIF2a. All Magnification is 40×.

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Figure 2.

Proposed model of neutrophilic amplification in pyoderma gangrenosum. A patient with a defect in suppression of neutrophil activation is attracted to the site of cutaneous injury by the innate immune system. The neutrophil, which does not resolve inflammation upon appropriate physiologic stimuli, continues to recruit additional neutrophils, amplifying the pathologic process.