Classic Ulcerative Pyoderma Gangrenosum Is A T Cell Mediated Disease Targeting Follicular Adnexal Structures: A Hypothesis Based On Molecular And Clinicopathologic Studies

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INTRODUCTION

Classic pyoderma gangrenosum (PG) is an ulcerative neutrophilic dermatosis that is the most common skin disease associated with inflammatory bowel disease (IBD). Diagnosis of PG is extremely challenging and treatment options are limited. Although it is most commonly thought to be a neutrophilic dermatosis, PG pathophysiology is actually poorly understood. The dominant hypothesis is that altered innate immunity leads to systemic autoinflammation (Aronozono et al., 2012).

An alternative view is that T cells are involved in PG pathophysiology (Antiga et al., 2011), yet there are no current theories on autoimmune targets. Herein we attempt to gain insight into the pathophysiology of PG by characterizing the cellular and molecular events prior to ulcer formation and after ulcer healing.

MATERIALS & METHODS

Ten patients with history of classic ulcerative PG were included. All ten patients were asked about patterns of ulceration (figure 1). Four PG patients with well-controlled disease underwent punch biopsies of PG scars and adjacent normal skin (“normal skin”) for immunohistochemical analyses. Hyperbiotic scars from healthy patients were used as an additional control ("control scar.") Scars from patients with biopsy-proven discoid lupus were also obtained to additionally control for post-inflammatory changes ("discoid scar.").

Diagnosis of PG was verified by more than one board-certified dermatologist based on clinical history, physical examination findings, and biopsy findings.

Immunohistochemistry:

Skin biopsies were paraffin-embbeded, and 5 µm sections were stained. Biopsies were stained with H&E and Masson’s trichrome. Glimas stain was used to assess prevalence of mast cells. Immunohistochemical stains were performed with antibodies directed against markers listed in Figure 2. Differences in histological score are shown in Figure 3.

Gene expression analysis and qRT-PCR array:

Differentially expressed genes of immunologic significance were identified in early pre-ulcerative PG capillaries versus normal skin by quantitative real-time PCR (qRT-PCR) array. Tissue samples were stabilized by RNAlater addition. Total RNA was extracted using RNeasy plus mini kit and the quantity and integrity of RNA was determined by fluorometry and 2200 TapeStation, respectively. Total RNA was reverse-transcribed to cDNA using (ScriptBio) and qRT-PCR was performed using customized PrimPCR plates from Bio-Rad with GAPDH, TBP1, HPRT1 and reference genes following the Minimum information for Publication of Quantitative Real-Time PCR Experiments (MIQE) Guidelines. Using a custom qRT-PCR array with validated primer sets, ScisssorsUniversal Universal SYBR Green Supermix, and the CFX96 Touch Real-Time PCR Detection System, differential gene expression analysis and corresponding statistical analysis was performed.

Statistical Analysis:

Differences in histological scores were assessed with one-way analysis of variance (ANOVA), followed by Bonferroni post hoc tests. SigmaStat 4.0 was used to perform analyses. Bio-Rad CFX Manager was used to perform differential cytokine gene expression analysis and corresponding statistical analysis (Bio-Rad Laboratories, Hercules, CA).

RESULTS

Figure 1. Patterns of ulcer formation in classic pyoderma gangrenosum. (A) PG scars do not exhibit inflammatory changes, ulceration, and limited scar formation over the ulcer field. (B) PG scars are shown after ulceration and an area strom to be devoid of inflammatory changes. (C) Ulcer PG appears to reflect the clinical surface of the lesion. (D) Ulcer PG appears to reflect the clinical surface of the lesion.

Figure 2. Histological scoring. A) Neutrophils are stained brown in H&E slides. Neutrophils are increased in PG compared to normal skin and discoid scar. B) Mast cells are stained pink in Glimas stain. Mast cell infiltration is increased in PG compared to normal skin and discoid scar. C) Glimas stain used to assess prevalence of mast cells. Mast cell infiltration is increased in PG compared to normal skin and discoid scar. D) Scars stained with Giemsa stain to assess prevalence of mast cells. Mast cell infiltration is increased in PG compared to normal skin and discoid scar.

Figure 3. Pyoderma gangrenosum, scoring scale. A) H&E stained section of PG lesion showing dense neutrophilic infiltrate. B) Glimas stain showing increased mast cell infiltration in PG lesions. C) Immunohistochemistry showing increased T cells in PG lesions. D) Immunohistochemistry showing increased CD68+ cells in PG lesions.

Figure 4. Expression patterns of regulated genes listed in tables 1 and 2. A) PG tissue (blue, dark blue and black line) is compared to normal skin (red, orange and marroon line) and discoid scar (green, red and light blue line). B) qRT-PCR array showing differences in gene expression.

Figure 5. Pyoderma gangrenosum tissue immunohistochemistry and gene expression analysis. A) Neutrophils are stained brown in H&E slides. Neutrophils are increased in PG compared to normal skin and discoid scar. B) Mast cells are stained pink in Glimas stain. Mast cell infiltration is increased in PG compared to normal skin and discoid scar. C) Glimas stain used to assess prevalence of mast cells. Mast cell infiltration is increased in PG compared to normal skin and discoid scar. D) Scars stained with Giemsa stain to assess prevalence of mast cells. Mast cell infiltration is increased in PG compared to normal skin and discoid scar.

CONCLUSIONS

All PG patients reported that healed sites of previous ulceration are refractory to re-ulceration. Simultaneous biopsies of healed and uninvolved skin triggered ulceration only in the latter. PG scar lesions and body areas devoid of follicular adnexal structures are resistant to development of PG ulcers.

On immunohistochemistry, healed PG scar showed complete loss of pilosebaceous units, which were present in normal skin, and to a lesser extent in control scars, and discoid scars.

Early PG capillaries showed perivascular and periadnexal T cell infiltrates, rather than neutrophils. These early inflammatory events were dominated by increased expression of CXCL9, CXCL10, CXCL11, IL-8, IL-17, IFNγ and IL-36γ, and transcription factors consistent with Th1 phenotype.

PG results from aberrant cytokine expression and autoactive T cells directed against follicular adnexal structures.

REFERENCES


Antiga, E., Maglie, R., Volpi, W., Bianchi, B., Bert, E., Marzano, A.V., et al. in press 1-related molecules as well as T15 are hyperexpressed in the skin lesions of patients with pyoderma gangrenosum. Clinical & Experimental Immunology, n/a: n/a-010.1111/cei.12989.


ACKNOWLEDGEMENTS AND CONTACT

This work was supported by a grant from the NIH (#1DP2OD018752-01) awarded to EM. EM was also supported by career awards from the Burroughs Wellcome Fund and the Howard Hughes Medical Institute. We thank Dr. Jennifer Urban for taking photos of PG affecting the hands (figure 1) and for her outstanding patient care. We also thank Dr. Farzam Gorouhi for performing one of the PG biopsies and for his outstanding care of one of our patients with discoid lupus.

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