OBSERVATION

Interleukin 23 Expression in Pyoderma Gangrenosum and Targeted Therapy With Ustekinumab

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Background: Interleukin (IL)-23 is involved in the pathogenesis of the chronic inflammatory Crohn disease. Pyoderma gangrenosum (PG) is often associated with and can even be the first manifestation of this disease and has abundant neutrophilic infiltration. Because IL-23 plays a critical role in driving inflammation associated with IL-17 production and especially neutrophil recruitment, we suspect that PG might be driven by a pathogenetic mechanism similar to that of inflammatory bowel diseases or psoriasis.

Observations: Tissue sample analysis showed highly elevated expression of IL-23 on both transcriptional and protein level in a recalcitrant PG lesion. On the basis on these data, a treatment targeting the p40 subunit of the heterodimeric IL-23 with the monoclonal antibody ustekinumab was started. Therapy with ustekinumab resulted in a significant decrease of IL-23 expression in PG and healing after 14 weeks of treatment. No relapse occurred in a 6-month follow-up period.

Conclusions: Our data provide evidence of an IL-23–dominated inflammatory infiltrate in PG. This might specify a new concept for PG pathophysiology and suggests a possibility for using ustekinumab as a therapeutic agent in this disease.

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Pyoderma gangrenosum (PG) is a rare disease (a retrospective analysis of medical records revealed 180 cases diagnosed at the Mayo Clinic, Rochester, Minnesota, over a 53-year period); 86 patients with PG were evaluated and treated over 12 years at the Wake Forest University School of Medicine, Winston-Salem, North Carolina, and the University of Louisville, Louisville, Kentucky, and there is currently no gold standard of treatment or published algorithms for choice of therapy. The clinical features of PG have been well characterized; however, little is known about its underlying pathogenetic and immunologic alterations. In 25% to 50% of the patients, the existence of an underlying immunologic abnormality is currently presumed, given the frequent association of PG with systemic diseases with a suspected autoimmune or autoinflammatory pathogenesis. Intestinal bowel disease, arthritis, paraproteinemia, and hematological malignancy have been reported as associated conditions, but none of these findings has been demonstrated consistently and it is not clear whether there is a causal relation.

Today, PG can be classified into 4 main clinical-pathological variants: ulcerative, pustular, bullous, and vegetative PG. Indeed, the more frequently present ulcerative PG is either idiopathic or associated mainly with intestinal bowel disease.

Because interleukin (IL)-23 is thought to be a key factor in the pathogenesis of immune disorders like psoriasis and inflammatory bowel conditions, we investigated the immunological profile of a PG lesion regarding IL-23.

Report of a Case

A 37-year-old woman presented with recalcitrant PG on the right leg. The first signs of PG had started 4 weeks earlier, and systemic treatment with prednisolone (50 mg/d) in combination with topical tacrolimus for 3 weeks had been unsuccessful. Clinical examination revealed a growing skin ulcer with sharply demarcated bluish undermined margins on the left pretilial region. At present, there was no sign of associated autoimmune disease. Two diagnostic skin biopsy specimens were obtained; one was embedded in paraffin and the other
was cryopreserved. Subsequently, total RNA from the shock-frozen samples was isolated and real-time polymerase chain reaction was performed. Interestingly, the tissue sample analysis showed notable elevated expression of IL23A in the PG lesion, compared with biopsy specimens from healthy skin (Figure 1A). The expression of IL17A and IL12A was further analyzed, but no noteworthy differences could be observed (data not shown). Histological analysis of the paraffin-embedded skin biopsy specimen by hematoxylin-eosin stain revealed PG characteristics such as numerous neutrophils and infundibular cysts (Figure 1B). In addition, we performed triple-color immunofluorescence, using anti–IL-23p19 and anti–MHC II primary antibodies together with Cy3- and Cy5-conjugated secondary antibodies. Nuclei were stained with green-fluorescent dye (YO-PRO; Invitrogen Corporation, Carlsbad, California), and detection of specific staining was done by confocal laser scanning microscopy. As suggested from the real-time polymerase chain reaction data, the PG lesion showed IL-23 protein (Figure 1C). Immunofluorescence analysis of healthy skin as a negative control did not reveal any IL-23 (data not shown).

On the basis of these data and having in mind the notoriously problematic treatment of PG, we decided to start a specific treatment, targeting IL-23. We commenced systemic treatment with ustekinumab, an anti–IL-12/IL-23p40 monoclonal antibody, at a dose of 45 mg administered twice on week 0 and week 4. The weight of the patient was approximately 70 kg. The topical application of tacrolimus was sustained. The treatment was tolerated well with no adverse effects. Eight weeks after the treatment start with ustekinumab, we observed cleaning of the bluish margins, and the PG lesion scaled down from 36 cm to 20 cm size. After 14 weeks of treatment, the PG had healed completely (Figure 2). The follow-up examinations up to 6 months after administration of the last ustekinumab-dose showed complete resolution of the PG and no relapse in the absence of ustekinumab.

**COMMENT**

Pyoderma gangrenosum is a severe inflammatory disease of the skin and its treatment is challenging and often unsuccessful. As the pathophysiologic mechanism of PG is mainly unknown, treatment concepts for PG are based on established regimen for inflammatory and autoimmune diseases. Occasional case reports and small case-series demonstrate a role for systemic glucocorticosteroids, cyclosporine, mycophenolate mofetil, thalidomide, intravenous immunoglobulin, anti–tumor necrosis factor therapy and other immunosuppressive and immunomodulating agents as treatment modalities for PG. However, lack of prospective randomized controlled trials and the unclear pathophysiologic mechanism hamper the progress for consistent concepts of PG treatment.

Herein, we provide the first evidence to our knowledge of an IL-23 dominated inflammatory infiltrate in PG. The T-helper 17 (T\textsubscript{h}17)-driving cytokine and consists of 2 subunits p19 and p40, the p40 subunit being shared with the T\textsubscript{h}1-driving IL-12. As ustekinumab specifically targets the common p40 subunit, its therapeutic efficacy might either directly affect...
the IL-12 and/or IL-23–producing dendritic cells or might result from an impact on the inflammatory Th1 and/or Th17 cells, respectively. The observed abundance of IL-23 and the absence of IL-12 in the PG lesion suggest that ustekinumab mainly affects the IL-23/Th17 arm of the immune response, at least in PG. We believe that further investigations will reveal the relevance of this finding for other inflammatory and autoimmune conditions. Interestingly, in some of these conditions (eg, psoriasis) antibodies against the IL-12/IL-23p40 subunit proved to be efficacious, yet the therapeutic effect could not be attributed to one single cytokine target until now.

The role of IL-23 in the pathogenesis of PG has not been previously addressed. Our report provides important insight into the pathogenesis of PG, marks IL-23 as a novel target for specific treatment for this disease, and demonstrates the clinical efficacy of ustekinumab in PG. Based on these pioneer data, further large-scale clinical trials are needed to assess the exact effectiveness and safety of blocking IL-12/IL-23p40 as a treatment option in PG.

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