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(Citation)
British Journal of Dermatology,157(6):1279-1281

(Issue Date)
2007-10-04

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90000846

**Pyoderma gangrenosum and interleukin-8**

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Sir,

Interleukin (IL)-8 is a potent chemotactic polypeptide for neutrophils.[1] We have previously demonstrated that high levels of IL-8 are immunohistochemically detectable in dermal fibroblasts from ulcers of patients with pyoderma gangrenosum.[2] Furthermore, overexpression of IL-8 using an adenovirus vector in the skin of severe combined immunodeficiency mice has been shown to result in the development of skin ulcers resembling pyoderma gangrenosum.[2] To clarify the level and mode (constitutive or transient) of IL-8 production in fibroblasts from a patient with pyoderma gangrenosum, the present study isolated and cultured fibroblasts from a patient with pyoderma gangrenosum, and measured IL-8 levels in supernatants. Serum IL-8 was also measured.

An 81-year-old woman presented with a 4-year history of diarrhoea. A painful skin ulcer had developed in the right breast 2 months prior to examination and enlarged rapidly. In addition, loss of appetite and bloody stools began from 1 month prior to examination. Ulcerative colitis was diagnosed in the Department of Internal Medicine at Higashi Kobe Hospital and she was admitted to hospital. She had not received any pharmacotherapies prior to admission and was treated using mesalazine alone (2250 mg/day for the first 3 weeks, 1500 mg/day thereafter). The patient was referred to the Department of Dermatology in the same hospital for diagnosis of the skin ulcer. The ulcer measured 12 × 10 cm with an erythematous, raised edge on the right breast (Fig. 1A). The ulcer base was irregular and displayed a granular appearance. After obtaining informed consent, skin biopsies were taken from both the edge of the ulcer and uninvolved skin of the left upper arm for the purposes of diagnosis and cultivation of fibroblasts. Biopsy of the ulcer showed non-specific changes with a dermal infiltrate comprising neutrophils, and to a lesser extent, histiocytes and mononuclear lymphocytic cells (Fig. 1B). Neutrophils were also identified in blood vessels. Extravasation of erythrocytes was present, but no evidence of vasculitis was apparent. Biopsy of

uninvolved skin showed normal skin with no infiltration of neutrophils (data not shown). Clinical and histopathological findings led to a diagnosis of pyoderma gangrenosum and the skin lesion was treated using topical sulfadiazine ointment. Mesalazine therapy led to rapid improvements in both ulcerative colitis and pyoderma gangrenosum and the skin ulcer was almost eradicated leaving scar tissue after 6 weeks of mesalazine therapy.

Fibroblasts from the pyoderma gangrenosum ulcer and uninvolved skin were isolated and cultured as described previously.[3] IL-8 levels in supernatants of cultured fibroblasts were measured by ELISA, as described previously [2] at several time points after almost complete elimination of contamination by other types of cells. Fibroblasts from the ulcer produced high levels of IL-8 in the early days of primary culture (Table 1). However, IL-8 production decreased rapidly and became undetectable by 27 days after the start of primary culture. IL-8 levels in the supernatants of fibroblasts from uninvolved skin were below the level of detection at all time points up to 41 days after the start of primary culture. Serum IL-8 concentrations were also measured in the patient at several time points after initiating mesalazine therapy (Table 2). Serum IL-8 levels were elevated in the early days of treatment, but fell below detectable levels by 40 days after starting treatment.

One of the characteristics of pyoderma gangrenosum is massive infiltration of neutrophils in the dermis.[4] Certain factors that induce neutrophil accumulation may be mediators of this effect. IL-8 could represent one such factor and our previous report [2] suggested that IL-8 produced by dermal fibroblasts plays a part in the pathogenesis of this disease. The present study showed that fibroblasts from the ulcer of pyoderma gangrenosum produced high levels of IL-8 *in vitro*, whereas fibroblasts from uninvolved skin did not. Production of IL-8 by fibroblasts from the skin lesion decreased rapidly as cultivation proceeded, indicating that production of the chemokine by fibroblasts is not constitutive. In addition, IL-8 production from uninvolved skin was undetectable levels, showing that IL-8 is locally produced in fibroblasts in the skin lesion. IL-8 production is

not constitutive in normal fibroblasts *in vitro*, similar to other types of normal cells,[1] and is induced following stimulation by pro-inflammatory cytokines such as IL-1 or tumour necrosis factor α.[5] Increased production of IL-8 in fibroblasts from pyoderma gangrenosum may be induced by local stimulation with such pro-inflammatory cytokines.

Concentrations of IL-8 were elevated in serum from the patient in the initial days of treatment, returning to undetectable levels after the skin lesion had healed. Saito *et al*. have also reported a case of pyoderma gangrenosum with increased levels of serum IL-8.[6] At present, pyoderma gangrenosum is a diagnosis of exclusion[7] and there is much room for diagnostic error.[4,7] Conversely, serum IL-8 levels have been shown to be high in some patients with ulcerative colitis.[8] Serum IL-8 levels in the present patient may thus reflect IL-8 from both pyoderma gangrenosum and ulcerative colitis lesions. Measuring serum IL-8 levels may be helpful for diagnosis if serum IL-8 levels can be shown to be high in the serum of patients with pyoderma gangrenosum.

One of the mechanisms of action for mesalazine is down-regulation of IL-8 production from cells.[9,10] This fact and our previous [2] and present studies strongly suggest the involvement of IL-8 in the pathogenesis of pyoderma gangrenosum. Elucidation of the mechanisms underlying high production of IL-8 in the fibroblasts of patients with pyoderma gangrenosum will be helpful in understanding the pathophysiology of this disease.

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rheumatoid arthritic synovial tissue chemokine production. *Exp Mol Pathol* 2002; **73:** 84-92.

**Figure legends**

**Figure 1: A)** Clinical appearance of pyoderma gangrenosum ulcer. **B)** Histopathological findings. Non-specific dermal infiltration of neutrophils is seen, along with histiocytes and mononuclear lymphocytic cells to a lesser extent. Neutrophils are also observed in blood vessels.

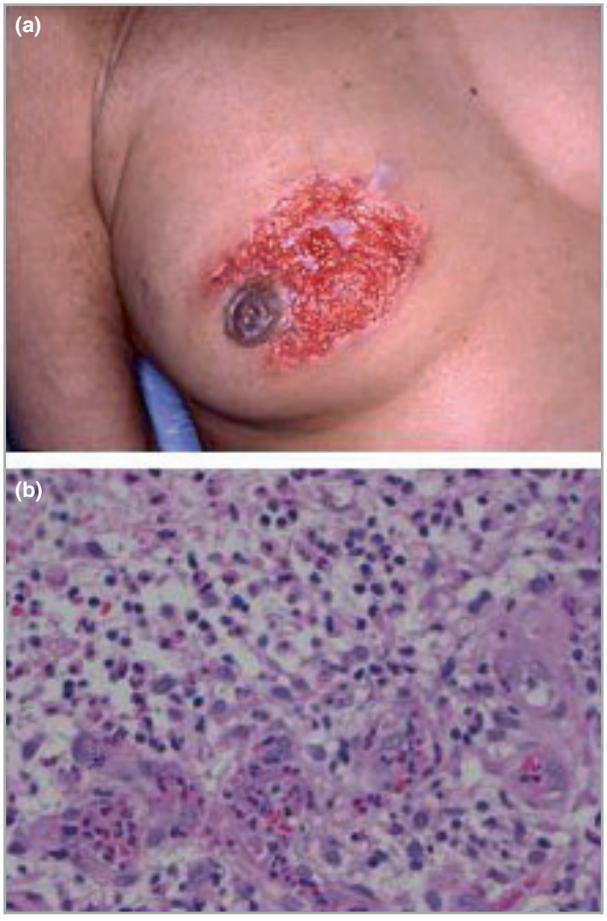

**Table 1** Interleukin (IL)-8 levels in cultured fibroblasts from a biopsy specimen from the edge of the ulcer

| Time after primary culture (days) | 13 | 16 | 21 | 25 | 34 | 41 |
|---|---|---|---|---|---|---|
| IL-8 (pg per $1 \times 10^6$ cells in 24 h) in the supernatant[a] | 47 | 35 | 2·7 | UD | UD | UD |

[a]IL-8 protein content in the culture medium was measured using an enzyme-linked immunosorbent assay kit (Biosource, Camarillo, CA, U.S.A.) according to the manufacturer's instruction. UD, under the level of detection.

**Table 2** Serum levels of interleukin (IL)-8 after the start of mesalazine therapy

| Time after start of therapy (days) | 15 | 21 | 40 | 60 |
|---|---|---|---|---|
| IL-8 (pg $mL^{-1}$)[a] | 224 | 188 | UD | UD |

[a]IL-8 protein content in the serum was measured using an enzyme-linked immunosorbent assay kit (Biosource, Camarillo, CA, U.S.A.) according to the manufacturer's instruction. UD, under the level of detection.