Pyoderma gangrenosum after trauma in a dog

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NOTE Internal Medicine

Pyoderma gangrenosum (PG) is a rare, ulcerative, neutrophilic dermatosis in humans [1]. Although the disease is idiopathic in some patients, the high frequency of other immune-mediated comorbidities supports an immune-mediated pathogenesis for this disease [3]. In addition, the phenomenon of pathergy is reported in about 30% of human patients with PG [3]. Pathergy is defined as the onset of skin lesions at the site of trauma, such as cuts, needlestick injuries or surgery [5]. This phenomenon, thought to reflect abnormal neutrophil function [4], is one of the criteria for the diagnosis of Behçet’s disease (the pathergy test) [7].

PG is likewise uncommon in dogs, with only two previous reports. One described development of PG after the diagnosis of non-erosive idiopathic polyarthritis [2], while the other noted two cases of PG with no apparent underlying disorder [17]. Little information has been accumulated regarding the characteristics of PG in dogs; therefore, consensus about treatment for this disease is lacking.

We describe the case of a dog that developed PG after trauma in which pathergy was suspected, with a good response to medical treatment with prednisolone alone. Moreover, we evaluated transcription levels of cytokine mRNA in lesional skin before and after treatment from this dog.

A 12-year-old male entire Miniature Pinscher was brought in to a night-time animal first-aid center with lethargy after being entrusted to a boarding kennel for 9 days. The dog was treated for dehydration, hypothermia (36.6°C) and hypoglycemia. The dog was then brought to our hospital the following day (day 1), upon which physical examination revealed exorication at various body sites, including the right eyelid, pinnae, neck and extremities. Swelling of the right eyelid was also evident (Fig. 1A). Body temperature was 39.3°C. The owner and staff at the boarding kennel could not explain the cause of skin lesions, but no external factors, such as heat burn, cold exposure and chemical reaction, were reported. Previous medical histories of this dog included intermittent corticosteroids as prescribed by other facilities for pruritus by suspected allergic dermatitis.

A complete blood count showed neutrophilia (26,200/µl, reference range 3,000–11,500/µl). Laboratory data indicated increased C-reactive protein concentration (CRP; 7.0 mg/dl, reference range 0–0.9 mg/dl). Urinalysis, thoracic and abdominal radiography, and abdominal ultrasonography revealed unremarkable findings.

Although the causes of lethargy and skin lesions were unknown, the dog was hospitalized with supportive therapy. In addition to intravenous fluid infusion with acetate Ringer’s solution (Solacel; Terumo, Tokyo, Japan) and maintenance fluid (Soldem 3A; Terumo), it was initiated oral cephalaxin (Larixin; Toyama Chemical, Tokyo, Japan) at 20 mg/kg twice daily for 21 days, enrofloxacin (Baytril; Bayer, Tokyo, Japan) at 7 mg/kg once daily for 21 days and firocoxib (Previcox; Merial, Tokyo, Japan) at 10 mg/kg once daily for potential bacterial infection and inflammation. Four days after initial presentation, the condition and blood abnormalities of the dog had improved; the dog was therefore discharged.

On day 9, the dog was brought back due to skin lesions with erosion on the right eyelid and cutaneous ulcers with necrotic eschar on the pinnae, neck and extremities. Cytology of a lesion showed sterile suppurative inflammation. Over the next several days, skin lesions progressively enlarged, and CRP again increased (8.7 mg/dl). Endocrine testing including thyroid function test and adrenocorticotroic hormone-stimulation test was within reference ranges. Antinuclear antibody test was also negative. On day 13, skin biopsies were performed (Fig. 1B). Histopathological
examination revealed necrosis of the epidermis, dermis and follicles, and moderate to marked neutrophilic infiltration of ulcer sites, perivascular and muscular layers, with no microorganisms, vasculitis, neoplasia or thrombus (Fig. 2). No infectious agents were identified using specific stains, such as periodic acid-Schiff, Ziehl-Neelsen, Grocott or Gram staining. Firocoxib was then replaced with prednisolone (Predonine; Shionogi, Osaka, Japan) at 1 mg/kg twice daily on the basis of suspected sterile neutrophilic dermatosis. On day 21, desquamation of necrotic skin was seen (Fig. 1C) along with decreased CRP (<0.9 mg/dl), and cephalexin and enrofloxacin were terminated. On day 49, skin lesions had almost completely resolved (Fig. 1D). Prednisolone dose was tapered over 4 months and then terminated, and no recurrence has since been identified. This case was diagnosed as PG based on the proposed diagnostic criteria for human PG, which require the satisfaction of two major criteria (including rapid progression of a painful, necrolytic cutaneous ulcer with an irregular, violaceous and undermined border, and exclusion of other causes of cutaneous ulceration) and at least two minor criteria, including (a) a history suggestive of pathergy or a clinical finding of cribriform scarring, (b) systemic diseases associated with PG, (c) histopathologic findings (sterile dermal neutrophilia ± mixed inflammation ± lymphocytic vasculitis) or (d) treatment response (rapid response to systemic corticosteroid treatment) [18]. This case fulfilled many of the criteria, including progressive necrolytic ulcerations, exclusion of other causes of ulceration such as infection, vasculitis, neoplasia or thrombus, a history suggestive of pathergy, histologic findings of sterile dermal neutrophilia and rapid response to systemic corticosteroids.

A pair of skin biopsy samples was obtained from lesional skin before (day 13) and after (day 59) treatment. The sample of skin after treatment was obtained from the scar at the site of lesional skin. All skin samples were immediately submerged in RNA later (Qiagen, Valencia, CA, U.S.A.) and stored at −20°C for 117 days (skin before treatment) and for 71 days (skin after treatment) until the extraction of total RNA using an RNeasy Plus Mini Kit (Qiagen). Genomic
DNA was removed from samples with a TURBO DNA-free Kit (Applied Biosystems, Foster City, CA, U.S.A.) and then stored at −80°C for a day. Transcription of cytokine mRNA was quantified by two-step RT-PCR using a Thermal Cycler Dice® Real Time System TP800 (Takara Bio, Otsu, Japan) as described previously [6]. Total RNA (0.5 µg) was reverse-transcribed to cDNA using a PrimeScript™ RT Reagent Kit (Takara Bio). Quantitative real-time RT-PCR was performed using a SYBR® Premix Ex Taq™ II system (Takara Bio). CT (threshold cycle) values were determined using Thermal Cycler Dice Real Time System Multiplate RQ version 2.00 software (Takara Bio). All samples were examined in duplicate, and the mean value of ΔCT was calculated. Amounts of each mRNA transcription level (relative quantity) were calculated using 2-ΔCT, resulting in evaluation of samples as n-fold differences relative to that of the mean value of three reference genes. Primer pairs used in this dog are listed in Table 1. Primers for canine tumor necrosis factor (TNF)-α and interleukin (IL)-1β, IL-8 and IL-17A were designed using the freely available Primer 3 software (http://www.genome.wi.mit.edu/genome_software/other/primer3.html). The specificities of these primers were confirmed to amplify each target mRNA by sequential analysis of the PCR products using ABI Prism 3100 Genetic Analyzer (Applied Biosystems). For accurate quantification, three reference genes (CG14980, SDHA and TBP) were chosen (Table 1).

Figure 3 shows the relative quantities of each gene examined in this case and normal skin of healthy dogs (n=5). Transcription levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8 and IL-17A were higher in lesional skin before treatment than in skin after treatment or normal skin of healthy dogs. N.D.: not detected.
## Table 2. Characteristics of the present case, erythema multiforme, toxic epidermal necrolysis and vasculitis

| Cause                        | Types of eruptions | Distributions of eruptions | Types of inflammatory cells (histopathology) | Use of glucocorticoids | Prognosis                      |
|------------------------------|--------------------|----------------------------|---------------------------------------------|------------------------|-------------------------------|
| The present case             | Unknown            | Excoriation, erosion, ulcer| Eyelid, pinnae, neck, extremities           | Neutrophils (invasion of ulcer sites, perivascular and muscular layers) | Effective (monotherapy) Good (with no recurrence) |
| Erythema multiforme          | Drug reactions     | Erythematous macule, papule, urticarial plaque, vesicle, bulla, ulcer | Ventrum, mucocutaneous junctions, oral cavity, pinnae, footpads | Lymphocytes           | Possibly effective (in idiopathic, refractory, or severe cases) Relatively good (regressing by eliminating trigger factors in most cases) |
| Toxic epidermal necrolysis   | Drug reactions     | Erythematous macule, vesicle, bulla, ulcer | Body, mucosa (anywhere) | Lymphocytes (minimal inflammation) | Controversial Guarded to poor (no published mortality rate) |
| Vasculitis                   | Idiopathy (50% or more), vaccine reactions, drug reactions, neoplasia, infections, food | Purpura, erythema, bulla, ulcer, alopecia, wheal, papule, pustule | Pinnae and/or footpads (most common), paws, claws, lower legs, lips, tail, scrotum, oral mucosa | Neutrophils, eosinophils, mononuclear cells (invasion of vessel walls) | Possibly effective (mostly with other drugs such as pentoxifylline, sulfasalazine and dapsone) Relatively high recurrence rate |

Modified from Miller, W. H. Jr, Griffin, C. E. and Campbell, K. L. 2013. Autoimmune and immune-mediated dermatoses, pp. 432–500. In: Muller and Kirk’s Small Animal Dermatology, 7th ed. (Miller, W. H. Jr, Griffin, C. E. and Campbell, K. L. eds.), Elsevier, St. Louis., Scott, D. W. and Miller, W. H. 1999. Erythema multiforme in dogs and cats: literature review and case material from the Cornell University College of Veterinary Medicine (1988–96). Vet. Dermatol. 10: 297–309., and Nichols, P. R., Morris, D. O. and Beale, K. M. 2001. A retrospective study of canine and feline cutaneous vasculitis. Vet. Dermatol. 12: 255–264.

...ment than in skin after treatment or normal skin of healthy dogs.

Typically, lesions of human PG manifest most commonly on the lower extremities, as well as on the trunk, upper extremities, head and neck [3]. Primary lesions in humans are shown as painful nodules or pustules, either single or multiple, that evolve to superficial ulcers, often extending into the fat and fascia [15]. Primary lesions of three dogs in past reports have included facial swelling with ulcers on the muzzle [2], pustules and erosions on the body [17], and an ulcer and subcutaneous nodules on the neck and trunk [17]. Some differences are evident in the site of primary lesions between human PG and previously reported cases in dogs, with lesions of the lower extremities (hindlimbs) being uncommon in dogs, while lesions of the face were recognized in all dogs during the course of diseases [2, 17], as well as our case. In our case, skin lesions at multiple sites were excoriations rapidly turned into ulcers that progressively enlarged. The cause of the initial skin lesions is unclear, but the dog might have suffered trauma while in the kennel that might have caused the phenomenon of pathergy, although this possibility remains purely speculative. A history suggestive of pathergy, indicated as development of new lesions at the site of trauma, is included in the minor diagnostic criteria for PG [18].

Differential diagnoses for human PG may include vasculitis, malignancy, primary cutaneous infection, drug-induced or exogenous tissue injury [18]. Although diagnostic criteria have been proposed for human PG [18], none have been formally adopted [1, 5]. The diagnosis therefore remains primarily based on clinical features and exclusion [1]. Biopsy is mainly conducted to exclude other potential causes of ulceration, because of the non-specific histopathologic findings [15]. On the other hand, neutrophils can provide a cytologic hallmark of human PG [15]. Drug eruptions are suggested to most generally develop within 1–3 weeks of the initiation of treatment [10]. For this reason, drug eruptions could be possible, because this dog had received pharmacotherapy from initial presentation, including cephalaxin, enrofloxacin and firocoxib, which could potentially induce drug hypersensitivity [10, 19]. The lesions had worsened on day 9. Of these drugs, cephalaxin and enrofloxacin could result in erythema multiforme and toxic epidermal necrolysis as potential causes of skin necrosis and ulceration [10]. However, drug hypersensitivity with cephalaxin and enrofloxacin was ruled out, because of the continued prescription after amelioration. In addition, these cutaneous drug eruptions were also excluded based on the histopathological findings. Although skin biopsy in our case was conducted on day 13, which might have missed some findings, such as vasculitis, that could have existed at initial presentation, vasculitis was principally ruled out in this case based on the histopathology and clinical characteristics. The characteristics of the present case are compared with those of erythema multiforme, toxic epidermal necrolysis and vasculitis in Table 2 [10, 11, 16].

Immunosuppressive treatment with systemic corticosteroids and cyclosporine is considered to be the first-line therapy for this disease [13, 14]. In dogs, the few reports describing treatment for PG have included corticosteroid with cyclosporine [2, 17] and corticosteroid with azathioprine [17], with good initial response. Prednisone or prednisolone was prescribed to these dogs at initial doses of 0.75–1.0 mg/
kg twice daily, and no side effects were reported [2, 17]. In our case, prednisolone alone at an initial dose of 1.0 mg/kg twice daily was commenced to treat PG, achieving marked improvements without visible side effects. In a review of 24 PG cases in humans, 10 cases were treated using only corticosteroids [13]. The present case indicates that dogs with PG could be treated with corticosteroids alone, as in humans.

Pyoderma gangrenosum is classified within the group of neutrophilic dermatoses, however, pathophysiological and molecular characteristics remain largely unknown [4]. In humans, expression of cytokines, such as TNF-α, IL-1β, IL-8 and IL-17 (measured by immunohistochemistry or sandwich-based protein antibody array), was significantly higher in lesional skin of patients with PG than in normal controls [8, 9]. IL-8, a well-known chemotactic polypeptide for neutrophils, has been detected in dermal fibroblasts from ulcer sites of human PG, indicating that IL-8 plays an important role in the pathogenesis [12]. Moreover, overexpression of TNF-α at the lesion site during human PG indicates that TNF-α also has a significant role in the immunopathogenesis [8, 9]. This provides a principle for the use of anti-TNF-α drugs in human PG [1, 8, 9]. Other cytokines, such as IL-1β and IL-17, are also overexpressed in human PG and other neutrophilic dermatoses like Sweet’s syndrome, suggesting a potential role for the pathophysiology of the group of neutrophilic dermatoses [8, 9]. In our case, transcription levels of IL-1β, IL-8, TNF-α and IL-17A were all higher in lesional skin before treatment than in skin after treatment or normal skin of healthy dogs, implying that these cytokines were associated with the development of lesions in this dog, as in human PG [8, 9]. These results indicate that pathophysiological and molecular characteristics of canine PG might be similar with those of human PG, but further studies are needed to evaluate cytokine expression profiles in more cases.

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