

A case of ex vivo boosted immune cell therapy for chronic ulcerative dermatitis in a dog

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Abstract: A 13-year-old castrated male Shih Tzu was presented with recurrent otitis externa and ulcerative dermatitis. Previous investigations had failed to identify the cause, and the lesion was resistant to treatment. According to the histopathological examination, a tentative diagnosis of chronic ulcerative dermatitis with bacterial infection was made. To treat the patient, immune cell therapy with antibiotics was conducted every 2 weeks, for a total of six sessions. After treatment, the skin lesion resolved. No side effects were observed during therapy. To the best of our knowledge, this is the first case report of the use of immune cell therapy for canine ulcerative dermatitis.

Key words: Activated lymphocytes, dog, ex vivo boosted immune cells, ulcerative dermatitis

1. Introduction

Canine ulcerative dermatitis is induced by several causes. The main causes of this skin condition are infections including fungi, bacteria, and ectoparasites, along with autoimmune skin diseases such as pemphigoid and systemic lupus erythematosus (1–4). Differential diagnoses include neoplasia, thermal or chemical burns, and reactions to injections (5). Definitive diagnosis depends on excluding the differential diagnoses via history taking, dermatological examination, and characteristic histopathology of the skin samples (4).

Ex vivo boosted immune cell (EBIC) therapy is referred to as transfusion therapy with lymphokine-activated T killer cells (T-LAK) in human medicine (6). In EBIC therapy, the patient receives a number of activated immune cells isolated from the patient's peripheral blood mononuclear cells (PBMCs), which have been expanded ex vivo. In humans, this immunotherapy is commonly used to treat cancer patients (6). In dogs, several studies on T-LAK have been conducted and demonstrated reinforcement of the recipient's immunity with this therapy (7,8).

This case report describes canine chronic ulcerative dermatitis with resistance to several therapeutic agents that was finally controlled with immune cell therapy and antibiotics.

2. Case history

A 13-year-old castrated male Shih Tzu presenting with recurrent otitis externa and dermatitis was referred to our

clinic. He was treated with topical and systemic antibiotics and prednisolone for 9 weeks at a local hospital, but the treatments were ineffective.

Physical examination revealed bilateral skin lesions on the periocular, ear, axillary, groin, hind leg, and perianal regions. The skin lesions were severely erythematous and ulcerative with exudate and superficial crust (Figure 1). Cytology revealed severe cocci infection with massive neutrophils. The wood ramp test was negative. The complete blood count and serum biochemistry profile revealed low white blood cell count (3.13 U/L, reference interval [RI] = 6.70–18.3 U/L), high alkaline phosphatase (>3500 U/L, RI = 14–224 U/L), high gamma-glutamyl transferase (74 U/L, RI = 1–14 U/L), and high alanine aminotransferase (578 U/L, RI = 4–125 U/L). Antibiotic susceptibility results indicated that chloramphenicol and amoxicillin/clavulanic acid were suitable. Skin biopsy specimens were obtained from the right flank cutaneous lesions. Upon histopathological examination of the skin sample, the patient was diagnosed with chronic ulcerative dermatitis. Severe vesicular crust was observed in the upper area of the ulcer. Superficial crusting was evident at the surface and comprised neutrophils, keratin debris, and fibrin. Degenerated neutrophils infiltrated the superficial dermis, and lymphocytes and macrophages infiltrated the dermis and subcutis. Moth-eaten appearance was noted at the derma-epidermal junction. No acantholytic cells were observed. There was no evidence of neoplastic changes or autoimmune processes. Fungal stains were applied to the

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Figure 1. The ulcerative skin lesions before EBICs therapy: a) right ear, b) axillary region, c) perianal region, d) hind legs.

tissue, but no organisms were present. According to the antibiotic susceptibility results, the patient was treated with chloramphenicol (40 mg/kg; Helocetin; Chongkundang, Seoul, South Korea) and amoxicillin/clavulanic acid (20 mg/kg; Amocla; Kuhail, Chunan, South Korea) twice per day orally for 2 weeks. However, there was no response to antibiotics and no regression of skin lesions. The pruritus and exudate worsened. Following consultation with the patient's owner, the decision was made to conduct EBIC therapy with antibiotics. For autologous lymphocyte culture and expansion, a 10-mL blood sample was obtained from the patient's jugular vein every 2 weeks. The PBMCs were isolated from blood samples and were cultured and expanded *ex vivo* for 13 days. Interleukin-2 (IL-2) was used as a mitogen for lymphocyte activation. The isolation, culture, and expansion procedures for immune cells were obtained and modified from previous studies (7,8). The phenotypes of cells before and after processing were analyzed by flow cytometry, and the outcomes are presented in Figure 2. After expansion, the EBICs were suspended in 20 mL of standard saline and injected intravenously into the patient. The number of injected EBICs at each treatment session is provided in the Table. EBIC therapy was conducted every 2 weeks for a total of 6 sessions, and all skin lesions were resolved following treatment (Figure 3). During the therapy, no side effects were observed and blood examination revealed no abnormalities.

3. Results and discussion

Many skin diseases induce erosion and crusting secondary to intense inflammation and through self-trauma from pruritus (4). In recurrent or chronic

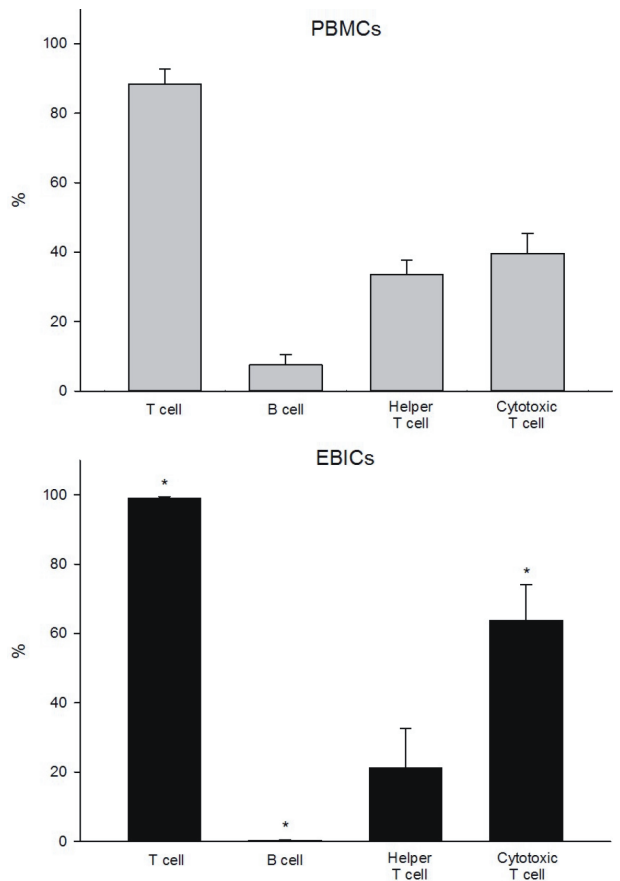
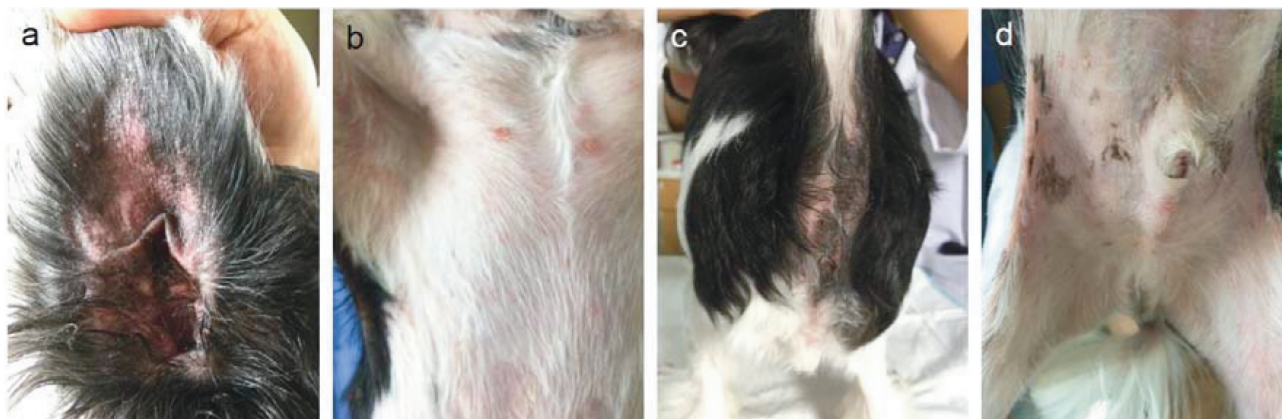


Figure 2. Phenotypes of immune cells before and after *ex vivo* culture. To clarify the cellular phenotype of PBMCs and EBICs, flow cytometric analysis was performed. The results are presented as mean \pm standard deviation. A comparison between PBMCs and EBICs was performed with the paired t-test. a) Phenotypes of PBMCs, b) phenotypes of EBICs. * $P < 0.05$.

Table. The characteristics of injected EBICs.

	1st	2nd	3rd	4th	5th	6th	Average
Expansion (fold)	18.4	8.8	143.3	335	240.4	390.9	240
Number of injected EBICs ($\times 10^8$ cells)	1.7	0.9	5.0	5.0	5.0	5.0	3.77

**Figure 3.** The skin after 6 sessions of EBICs therapy. All skin lesions resolved clearly. a) Right ear, b) axillary region, c) perianal region, d) hind legs.

ulcerative dermatitis, history taking, cytology, and skin biopsy should be performed to identify the causative factor (4). In this case, the patient had suffered recurrent external otitis and chronic ulcerative dermatitis. He had been previously treated with several systemic and topical antibiotics and antiinflammatory drugs with no response. Via careful examination, adverse cutaneous drug reaction, autoimmune skin disease, fungal infection, and neoplasia were eliminated as causative factors. Only severe cocci infection remained. Few studies have reported mucocutaneous pyoderma (MCP) in dogs (2,4). MCP is an ulcerative dermatosis of unknown etiology. Dogs with MCP present bilaterally symmetrical skin lesions with mild to moderately pruritic erythema and crusting. The locations of skin lesions include the anus, nares, periocular, or vulva/prepuce. Generally, MCP may respond well to antimicrobial therapy. However, in the present case, antimicrobial therapy was not effective, with the exception of the choice of drug based on cytology and antibiotic susceptibility test. Thus, EBIC therapy with antibiotic therapy was conducted.

In previous studies, administration of ex vivo activated lymphocytes in dogs could lead to resolution of inflammation and reinforce the recipient's immunity (7,8). In the present case, the number of cells increased by an average of 240-fold after culture and an average of 3.77×10^8 cells/session were injected into the patient. Flow cytometric analysis was performed to clarify the cellular phenotype of PBMCs and EBICs. The cells

comprised T lymphocytes ($CD3^+CD21^+$), B lymphocytes ($CD21^+$), helper T cells ($CD3^+CD4^+CD8^-$), and cytotoxic T cells ($CD3^+CD4^-CD8^+$). A comparison between PBMCs and EBICs was performed with the paired t-test. The proportion of T lymphocytes was predominant in both PBMCs (mean: 89.10%) and EBICs (mean: 99.04%). Following ex vivo expansion, the proportion of cytotoxic T cells increased significantly from 39.69% to 63.79%. These results were similar to those of previous studies (7,8). For a long time, cytotoxic T cells were considered to have the ability to kill tumor, allogenic, and virus-infected cells (9–11). In addition, several recent reports have demonstrated that cytotoxic T cells also have the ability to directly recognize and kill bacteria, parasites, and fungi (12). There were no studies regarding the correlation between T-cell therapy and infection in veterinary clinics. However, human PBMCs stimulated by IL-2 were found to inhibit the growth of microorganisms and T-cell therapy was considered a novel treatment for infectious disease (12).

In this case, during EBIC therapy, there was no change in patient activity or appetite and no adverse effects were observed. After 6 EBIC therapy sessions, all skin lesions had clearly resolved and there was no recurrence for 2 years. To the best of our knowledge, this is the first case report of the use of immune cell therapy for canine ulcerative dermatitis with bacterial infection. We could not explain the specific mechanism of action of EBIC therapy. However, we expect that this therapy could be used to treat refractory, antibiotic-resistant dermatitis.

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