A Case of Churg–Strauss Syndrome: Flow Cytometric Analysis of the Surface Activation Markers of Peripheral Eosinophils

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Churg–Strauss syndrome (CSS) is a complex multiple organ disease (1). The American College of Rheumatology has proposed 6 criteria for CSS classification, with the fulfilment of 4 criteria making a diagnosis of CSS (2). These criteria include asthma, eosinophilia, mononeuropathy or polyneuropathy, pulmonary infiltrates, paranasal sinus abnormality and extravascular eosinophils. Cutaneous manifestations are frequently observed in 40% of CSS patients. We report here a case of CSS presenting atypical cutaneous and subcutaneous manifestations of CSS with flow cytometric analysis of the peripheral eosinophils.

CASE REPORT

A 63-year-old man presented with an 18-month history of palpable purpura and scattered vesicles, which had not been diagnosed when he consulted another dermatologist. He had also had oedematous eyelids and conjunctivitis, which had been treated with corticosteroid eye-drops prescribed by an ophthalmologist. He developed a mandibular subcutaneous mass (Fig. 1a), which had been completely excised by an otolaryngologist 3 months before our examination. The histology of the mass showed dense inflammatory infiltrates with numerous eosinophils and a germinal centre arrangement of the lymphoid tissue. He began to experience asthma and allergic rhinitis 2 months before our examination. He was referred to our department because he developed another subcutaneous mass on the abdomen. He also presented with erythematous macules and palpable purpura on the lower legs one month after our first examination (Fig. 1b).

Laboratory investigations revealed normal urinalysis, prominent eosinophilia (64% of the total white blood cell count, 5,500 eosinophils/mm^3), a slightly elevated serum immunoglobulin E (IgE) (380 IU/ml), positive rheumatoid factor (37.2 IU/ml), positive antinuclear antibodies (40 titre) and negative antineutrophil cytoplasmic antibody values. Serum interleukin (IL)-5 and eosinophil cationic protein (ECP) levels were elevated (8.9 pg/ml, normal range; < 3.9 pg/ml: 91.5 mg/l, normal range; < 14.9 mg/l, respectively). Pulmonary function tests revealed an obstructive defect with significant bronchodilator reversibility, consistent with a diagnosis of asthma. Radiography and computed tomography of the chest, and echocardiogram were all normal. An examination of aspirated bone marrow revealed normocellular marrow.

We performed a total excision of the abdominal subcutaneous mass, and its histology showed lobular and septal panniculitis with numerous eosinophils (Fig. 1c). A skin biopsy from a purpuric papule on the lower leg revealed necrotizing vasculitis with pronounced inflammatory infiltrates with numerous eosinophils, fibrinoid deposit of the vessel wall, and extravascular granulomas (Fig. 1d). Direct immunofluorescence staining revealed the deposition of C3 in small vessels of the dermis. Differential diagnoses for hypereosinophilia including parasite infections were excluded. The clinical and pathological findings of a mandibular subcutaneous mass were similar to those

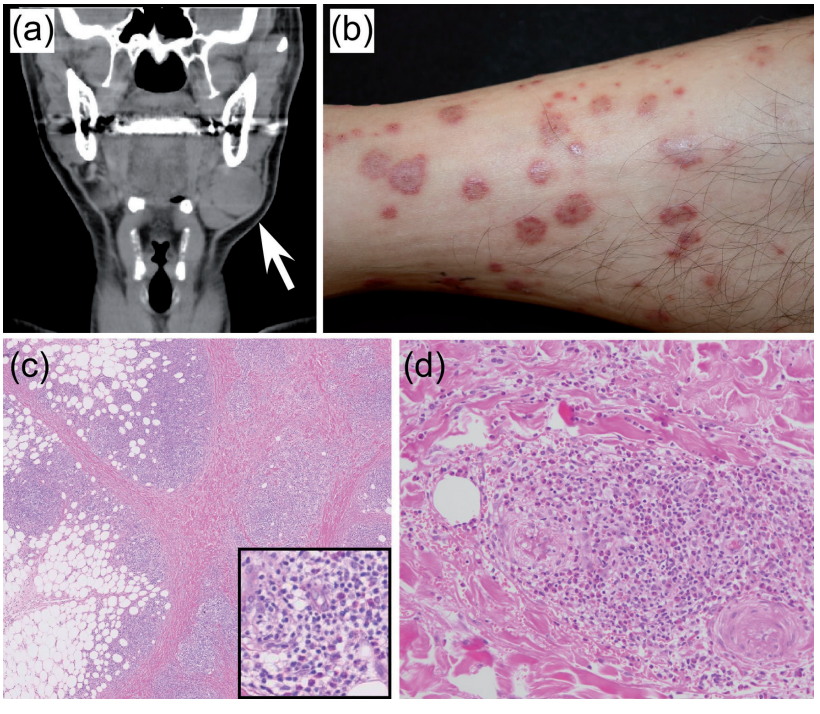

Fig. 1. (a) Computed tomography image of a mandibular subcutaneous mass. (b) Clinical picture of the lower leg. (c) Histology of an abdominal subcutaneous mass and (d) an erythematous papule on the lower leg. Haematoxylin and eosin; original magnification (c) ×25, (c, inset) ×200, and (d) ×200.

of Kimura's disease, and the abdominal subcutaneous mass histologically exhibited eosinophilic panniculitis. Therefore, this case was initially suspected to be Kimura's disease or eosinophilic panniculitis, both of which were ruled out because of the presence of necrotizing vasculitis and extravascular granulomas. We diagnosed our patient with CSS on the basis of histological cutaneous vasculitis, asthma, rhinitis and eosinophilia, which fulfilled the 4 criteria of ACR classification (2). Oral prednisolone (0.4 mg/kg/day) was thus commenced and the eruptions subsequently subsided and the laboratory findings improved (eosinophil, 9.8%; serum IL-5 levels, < 3.9 pg/ml; and serum ECP levels, 17.8 mg/l).

We also performed an evaluation of the eosinophil activation markers by flow cytometry before and after treatment. Eosinophils were identified with anti-human CCR3-phycoerythrin (R&D Systems, Minneapolis, MN, USA) positive and anti-human CD16-FITC (BD Biosciences, Franklin Lakes, NJ, USA) negative. The other antibodies of anti-human intercellular adhesion molecule-1 (ICAM-1), anti-human CD11b APC-eFluor 780, and anti-human CD69 were purchased from Cell Signaling Technology (Danvers, MA, USA), eBiosciences (San Diego, CA, USA) and R&D, respectively. Species-specific Alexa Fluor 647 (Life Technologies, Tokyo, Japan) were used for the second antibodies. We observed that the expression of ICAM-1 (Fig. S1a; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1384), CD11b (Fig. S1c) and CD69 (Fig. S1e) in this patient was enhanced compared with healthy subjects. In addition, this enhanced expression was normalized following treatment with oral corticosteroids (Fig. S1b, d, f). Informed consent was obtained from all subjects involved in this study, which was approved by the ethics committee of Kyoto University.

DISCUSSION

Palpable purpura of the extremities is the most common manifestation, affecting half of CSS patients with skin involvement. Less common manifestations include nodular lesions, livedo reticularis, ulcerations, bullous lesions, cutaneous infarcts, Raynaud's phenomenon and solar urticaria (3, 4). Our patient presented with palpable purpura, bullous lesions and subcutaneous masses, which were quite rare clinical manifestations in CSS.

IL-5 is the most important cytokine responsible for eosinophil differentiation and survival, and it was reported that elevated plasma IL-5 was detected in 30% of active CSS patients (5). In our patient, serum IL-5 concentration was elevated initially but decreased in response to oral corticosteroids. ECP has been reported to be increased and to correlate with disease activity in patients with CSS (6). In our patient, the serum ECP levels were also elevated, which suggested high eosinophil activity; these levels decreased after treatment.

We also analysed the surface expressions of ICAM-1, CD11b and CD69 on eosinophils in the peripheral blood. Several reports have suggested the importance of adhesion molecules, such as ICAM-1 and CD11b/CD18, in modulating the outcome of granulocyte macrophage colony-stimulating factor and IL-5 stimulation in human eosinophils, and ICAM-1 is readily induced by activation with proinflammatory cytokines (7). CD11b is one of the integrins that regulate the extravasation of eosinophils and is activated as a result of exposure to cytokines (8). CD69 is the activation antigen observed on the surface of purified blood eosinophils by stimulation with IL-5 (9). CD69 was highly expressed on the eosinophils of the peripheral blood of patients with active CSS (10). Eosinophils in the peripheral blood are activated to various degrees, possibly depending on cytokine stimulation. Although it remains unclear at present how valuable it is to measure activation markers of eosinophils in CSS, we assume that it will be beneficial to evaluate the activation status of CSS.

In summary, we report here a case of CSS presenting with atypical cutaneous and subcutaneous clinical manifestations with activated eosinophils in the blood, which may contribute to further elucidation of the pathophysiology of CSS.

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