

Case report

A case of episodic angioedema associated with blood eosinophilia: upregulated C5a receptor expression on eosinophils

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A 47-year-old woman was admitted to hospital complaining of swelling and pain of the extremities, accompanied by high fever and generalized erythema. Laboratory examination showed marked blood eosinophilia with elevation of IgM, IgE, and C-reactive protein. All autoantibodies examined were negative. The heart and lungs showed no untoward findings. Biopsies of the skin and muscle revealed cellular infiltration of eosinophils around small blood vessels. Quantitation of C5a receptor (C5aR) expression by flow cytometry using anti-C5aR antibody showed upregulated expression of C5aR on blood eosinophils but downregulated expression on neutrophils. The abnormal C5aR expression on eosinophils and neutrophils became normal after spontaneous resolution of symptoms and blood eosinophilia. The possibility that C5aR expression on granulocytes is related to the pathogenesis of this syndrome may be considered.

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The anaphylatoxin C5a activates human polymorphonuclear leukocytes through receptor-mediated processes (1). Eosinophils as well as neutrophils carry a receptor for C5a (C5aR) (2). We found no documentation on the expression of C5aR in clinical disorders. We report here changes in C5aR expression on leukocytes in a patient during episodic angioedema and blood eosinophilia.

Case report

A 47-year-old Japanese woman was admitted to the hospital with pain and swelling of the extremities and generalized erythema. The patient had been healthy until 2 weeks before when she noticed a rash on her hip. Red macules of varying size rapidly spread over the trunk and the extremities during the next few days. Nine days before admission, the patient began to have pain and swelling of the extremities, accompanied by a body temperature of 38°C. Seven days before admission, the patient visited our hospital, where hematologic examination showed a white blood cell count of 10 900/μl, with

88% neutrophils, 11% lymphocytes, and 1% eosinophils, and an erythrocyte sedimentation rate of 47 mm/h. The value of C-reactive protein (CRP) was 8.2 mg/dl. Blood chemistry findings were all within normal limits, except for slightly elevated values of serum alanine aminotransferase (ALT) (57 IU/l) and lactic dehydrogenase (LDH) (555 IU/l). Despite a prescription of diclofenac sodium, fever continued, and pain and swelling of the extremities became progressively worse until she was almost unable to change position. She was then admitted on 20 November 1995 (Fig. 1). On examination, erythema was present in the extremities and the trunk but became somewhat paler than before entry. The face was edematous, with reddish eyelids. Pea-sized lymph nodes were palpable in the bilateral submandibular regions. The oral cavity was clear, chest and the abdomen were normal, extremities were stiff and swollen, and pitting edema was present in the legs (Fig. 2A). The patient had difficulty in grasping things, raising arms, and walking because of pain and weakness. Sensation and the tendon reflexes were normal.

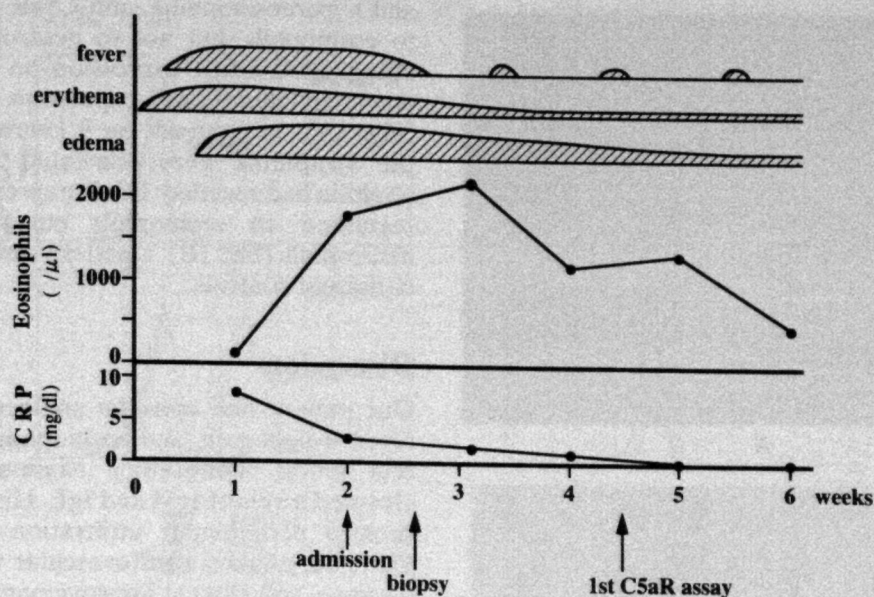


Fig. 1. Clinical course. CRP: C-reactive protein.

Hematologic examination showed a white blood cell count of $13000/\mu$ l, with 59% neutrophils, 23% lymphocytes, and 14% eosinophils; a red blood cell count of $3.53 \times 10^6/\mu$ l; hemoglobin of 10.1 g/dl; hematocrit of 32.2%; platelet count of $40.4 \times 10^4/\mu$ l; and erythrocyte sedimentation rate of 46 mm/h. Blood chemistry findings were as follows: total protein 6.1 g/dl (6.4–8.4), AST 11 IU/l (10–40), ALT 11 IU/l (5–40), LDH 431 IU/l (250–460), blood urea nitrogen 11 mg/dl (6–21), creatinine 0.9 mg/dl (0.6–1.5), creatine phosphokinase (CPK) 29 IU/l (32–180), and aldolase 9.4 mU/ml (1.9–4.7). The numbers in parentheses indicate (normal) standard values used in Japan. Electrolytes were normal. Serum electrophoresis showed albumin of 45.6%, α_1 -globulin of 6.0%, α_2 -globulin of 13.9%, β -globulin of 7.6%, and γ -globulin of 26.9%. The level of IgA was 246 mg/dl, IgG 1736 mg/dl, and IgM 1035 mg/dl. Complement examination showed C3 of 43 mg/dl (48–99), C4 of 15 mg/dl (14–52), and CH_{50} of 34 U/ml (30–50). The value of CRP was 7.6 mg/dl (<0.8). The Wassermann reaction and the *Treponema pallidum* hemagglutination test were negative. Serologic markers for hepatitis B and C viruses, Epstein-Barr virus, human T-lymphotropic virus type I, and human immunodeficiency virus were negative. The following autoantibodies were used for tests: rheumatoid arthritis factor, antinuclear antibody, anti-DNA antibody, anti-RNP antibody, anti-Jo-1 antibody, anti-Scl-70 antibody, and antineutrophilic cytoplasmic antibody, all of which were negative. The lymphocyte stimulation test for the drugs which had been prescribed, diclofenac

sodium, loxoprofen, and cefdinir, was consistently negative. An electrocardiogram gave a normal tracing. Radiographs of the chest and the abdomen showed no abnormalities, and ultrasonographic data on the heart and the abdomen were normal. Upper gastrointestinal endoscopy revealed superficial gastritis on the fornix. Four days after admission, biopsy specimens were taken of the erythematous skin lesions and muscle of the anterior aspects of the right thigh. Histologic analysis revealed edematous changes of connective tissue and eosinophilic infiltration, intermingled with mononuclear cells, around the small blood vessels (Fig. 2B).

Two weeks after admission, excoriation of the hands and feet was present with some diminution of the erythema. Edema and arthritis also improved gradually, but pain in elbows and knees remained. The peak of blood eosinophilia (2106/ μ l) was observed 8 days after admission but decreased with improvement of complaints as well as decreases in IgM, IgE, and CRP.

Material and methods

Analysis of C5aR expression on eosinophils and neutrophils

Granulocytes were isolated from heparin-anticoagulated venous blood from the patient by Ficoll density gradient centrifugation. Eosinophils and neutrophils were purified by negative selection with an immunomagnetic procedure with anti-CD16 (Nichirei, Tokyo) and anti-CD9 (Nichirei,

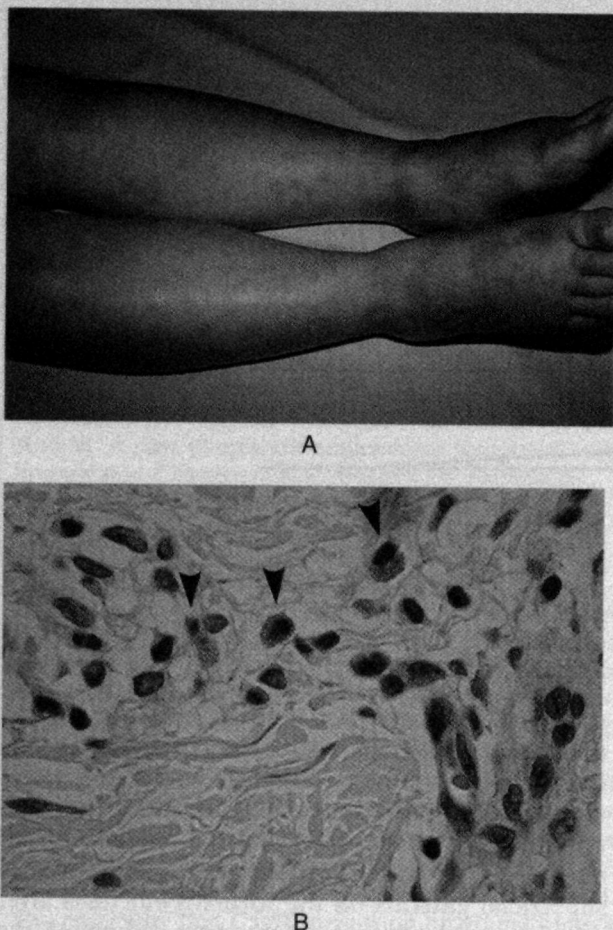


Fig. 2. A) On admission, extremities were stiff and swollen. Erythematous macules, associated with slight petechiae, were scattered on edematous skin of trunk and extremities. B) Micrograph of skin pieces from right thigh. Eosinophils (arrowheads) were scattered among mononuclear cell infiltrates around small blood vessels, where edematous changes were seen (HE $\times 1000$).

Tokyo) antibodies, respectively (3, 4). The resulting purity of eosinophils and neutrophils was about 100%. After isolation of eosinophils and neutrophils, C5aR expression was examined by flow cytometry, as described previously (5). In brief, cells (5×10^5) in staining buffer were incubated with anti-C5aR antibody or PBS for 30 min at 4°C , washed, and labeled with phycoerythrin-conjugated donkey F(ab')₂ fragments of antirabbit IgG (Cedar Lane, Ontario, Canada) for 30 min at 4°C . Cells (1×10^4) were analyzed by the flow cytometer (Epics XL-MCL; Coulter, Hialeah, FL, USA).

Results

Fig. 3 shows the results of single-color flow cytometric analysis of blood from the patient. On 9 December 1995, when the patient had symptoms

and hypereosinophilia, anti-C5aR antibody bound to eosinophils, but not to neutrophils, indicating upregulated C5aR expression on eosinophils but downregulated C5aR expression on neutrophils (Fig. 3A). In contrast, on 9 February 1996, when the symptoms were alleviated and hypereosinophilia had receded, C5aR expression was downregulated on eosinophils but upregulated on neutrophils (Fig. 3B), a pattern similar to that seen in normal controls.

Discussion

Our patient had episodic angioedema, erythema, fever, weight gain, and eosinophilia in the peripheral blood. Laboratory examinations showed elevated levels of IgM and IgE. Histologic analysis showed perivascular infiltration of eosinophils. She had neither cardiovascular nor pulmonary diseases, and clinical improvement was spontaneous. This case is consistent with the episodic angioedema associated with eosinophilia reported by Gleich et al. (6), an entity which can be distinguished from the hypereosinophilic syndrome. This seems to be the first report on this syndrome to examine C5aR expression on granulocytes.

Butterfield et al. reported that serum levels of interleukin (IL)-5 were elevated in patients with episodic angioedema and eosinophilia, suggesting that production of IL-5 may play an important role in the pathophysiology (7). In our patient, C5aR expression was upregulated on eosinophils, but this expression became normal in parallel with clinical improvement. C5a is a potent chemotactic factor for eosinophils and enhances vascular permeability (1). It has been reported that IL-3 and IL-5 prime eosinophils in normal subjects to produce leukotriene C₄ by sequential stimulation with C3a or C5a (8). Therefore, the upregulated C5aR expression on eosinophils and oversecretion of IL-5 may contribute to the pathogenesis of this syndrome, possibly due to the hyperresponsiveness of eosinophils to C5a.

In other cases of hypereosinophilia, we noted upregulation of C5aR expression on eosinophils in a patient with Churg-Strauss syndrome, but not in cases of bronchial asthma or allergic bronchopulmonary aspergillosis (unpublished data). These data suggest that C5aR upregulation does not occur in all cases with blood eosinophilia. While further cases must be compiled to assess the role of C5aR expression on granulocytes in clinical disorders with hypereosinophilia, our present data do suggest that C5aR overexpression on eosinophils may be related to the pathogenesis of this syndrome.

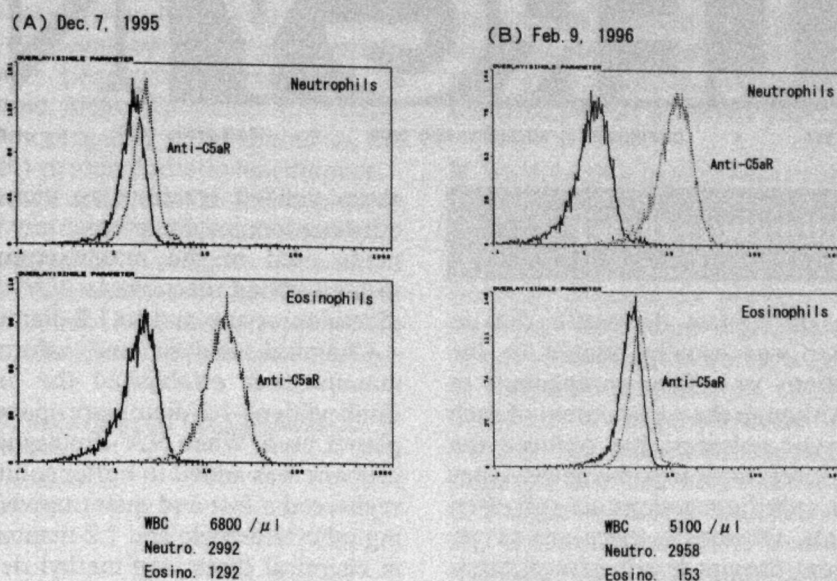


Fig. 3. Single-color flow cytometric analysis of blood from patient, collected on 7 December 1995 (A) and 9 February 1996 (B). Cells were stained with protein A-purified anti-C5aR plus phycoerythrin-conjugated secondary antibody (F(ab')₂ fragments of antirabbit IgG) (dotted line), or PBS plus secondary antibody which served as control (solid line). x-axis represents relative fluorescence intensity; y-axis, relative cell number. On 9 December 1995, anti-C5aR antibody bound to eosinophils, but not to neutrophils. In contrast, on 9 February 1996, anti-C5aR antibody did not bind to eosinophils but did bind to neutrophils.

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