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ORIGINAL ARTICLE

Serum Concentrations of Eosinophil Cationic Protein and Eosinophils of Patients with Kimura's Disease

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ABSTRACT

Background: To clarify the role of eosinophils in the pathogenesis of Kimura's disease and the values of measuring serum levels of eosinophil cationic protein (ECP) for monitoring disease activity might be very important, but there are few reports about this matter.

Methods: A total 14 serum and 7 tissue samples from patients with Kimura's disease were studied. The concentrations of ECP and cytokines (interleukin-4 (IL-4), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin 5 (IL-5)) in sera from patients with Kimura's disease were measured by enzyme-linked immunosorbent assay (ELISA). The density of eosinophils and the degree of activation of eosinophils in the tissue were also studied immunohistochemically.

Results: The concentration of ECP in sera from patients with Kimura's disease was significantly higher than that in the control group ($p < 0.05$). At the time of the remission, a significant decrease of ECP was observed. In interfollicular areas, most infiltrated eosinophils were positive for EG2 antibody (64.0–94.0%) and the mean percentage of EG2-positive eosinophils was 75.7%. The concentrations of IL-4, GM-CSF, and IL-5 in sera from patients with Kimura's disease were within normal ranges or below the detectable level in all sera examined.

Conclusions: Our findings suggest that eosinophils play an important role in the pathogenesis of Kimura's disease and ECP may be used as an additional parameter of disease activity.

KEY WORDS

EG-2, eosinophil cationic protein, eosinophils, Kimura's disease

INTRODUCTION

Kimura's disease is a rare benign disorder characterized by nodules found on the head and neck, associated with peripheral blood eosinophilia.[1] The nodules are located intradermally or subcutaneously. Histologically, the lesions comprise cellular and endothelial vascular elements. The lymphocytes present as focal infiltration with primary and secondary lymphoids. Infiltration of lymphocytes and histiocytes with a predominance of eosinophils is extensive.[2,3] While the etiology is not well understood, the disease is frequently associated with marked eosinophilia and an elevated serum level of IgE. Eosinophils are widely recognized as being proinflammatory cells and are considered to damage the tissues by releasing chemical mediators, such as eosinophil cationic protein (ECP). Recently, it has become possible to recognize eosinophil activation using the immunohistochemical marker, EG-2. To investigate the role of eosinophil in the pathogenesis of Kimura's disease, the concentrations of ECP in sera from patients with Kimura's disease were measured and the EG-2 positivity of eosinophils in the lesion was investigated immunohistochemically.

METHODS

SUBJECTS

Seven patients with Kimura's disease were referred to our department; six men and one woman aged from 21 to 72 years (mean age 43). The clinical details of these patients are shown in Table 1. The main

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Table 1 Clinical Features of patients with Kimura's disease

Case	Sex	Age	Location	Duration	Blood Eosinophil counts	EG-2 positive eosinophils
1	F	31	Parotid	5 yrs	1650 (19%)	71.4%
2	M	29	Parotid	5 yrs	1840 (23%)	79.8%
3	M	72	Parotid	10 yrs	1830 (30%)	72.4%
4	M	28	Neck	1 yrs	1512 (27%)	80.8%
5	M	58	Parotid	4 yrs	1250 (15%)	67.7%
6	M	62	Parotid	20 yrs	4800 (36%)	94.0%
7	M	21	Parotid	5 yrs	830 (10%)	64.0%

parotid: parotid gland

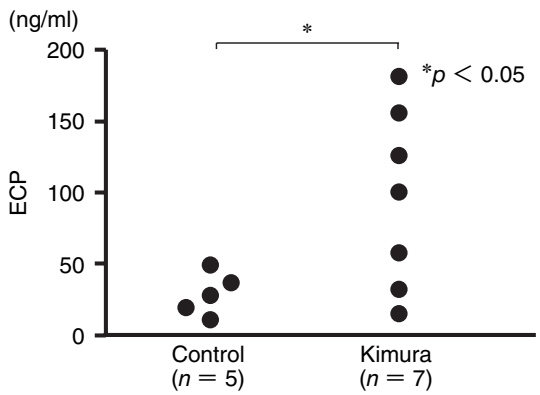

Fig. 1 Serum levels of ECP of patients with Kimura's disease and control subjects. The concentration of ECP in sera from patients with Kimura's disease was significantly higher than that of control.

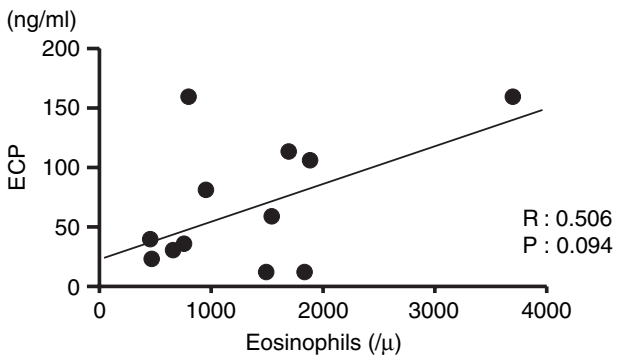

Fig. 2 The serum concentrations of ECP and the number of eosinophils. The serum ECP levels were not significantly correlated with the number of eosinophils from patients with Kimura's disease. ($p = 0.094$)

findings were irregular subcutaneous nodules (25–70 mm in diameter) that were itchy, sometimes fluctuated in size, and were tender. There was some discoloration of the overlying skin. The control group consisted of 5 (3 men and 2 women) healthy, non-allergic volunteers aged from 21 to 32 years (mean age 23.5).

IMMUNOHISTOCHEMICAL STUDY

Tumors and involved lymph nodes obtained from biopsy or surgically removed materials were fixed in 10% formalin for 1 or 2 days and dehydrated with a series of ethanol solutions, and then embedded in paraffin-wax. Sections of 3 mm in thickness were dewaxed in xylene and dehydrated. The sections were washed with 0.01 M phosphate-buffered saline (PBS) (pH 7.2) containing 0.15 M $NaCl_2$ and 0.01% Triton X-100 and incubated for 2 hours with the murine monoclonal anti-human ECP antibody, EG-1 or EG-2 (mouse IgG1, Pharmacia, Uppsala Sweden), which was diluted 1 : 10 in PBS containing 0.1% bovine serum albumin. Controls for nonspecific staining were incubated with 10 mg/ml of mouse IgG1 (DAKO, Glostrup, Denmark). Sections were washed and incubated with biotinylated rabbit antibody to mouse IgG, IgA, and IgM (Immunotech, Tokyo, Japan) for 1hour. The sections were incubated with the ABC reagent (Vectastain, ABC Elite; Vector Laboratories, Burlingame, CA), followed by 3, 3-diaminobenzidine (Dojin Chemicals, Kumamoto, Japan) as the chromogen. Finally, the slides were counterstained with hematoxylin.

METHODS OF ASSESSMENT OF SLIDES

Eosinophils containing no or only one EG-2 positive particle in cytoplasm were assessed as EG-2 negative cells. EG-2 positive eosinophils per total cells in a microscopical field (X400; as area = 0.0384 mm^2) were counted in the interfollicular area in each specimen. More than five areas were evaluated using an eyepiece graticule.

QUANTITATION OF ECP, GM-CSF, IL-4, AND IL-5

The measurement of serum ECP levels was performed using a Pharmacia ECP kit (Uppsala, Sweden), according to the manufacturer's directions for use. GM-CSF, IL-4, and IL-5 were also measured using a commercial enzyme-linked immunosorbent as-

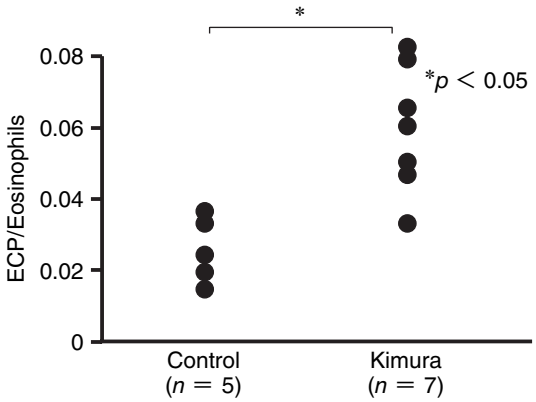

Fig. 3 The serum concentrations of ECP/the number of eosinophils. The serum ECP/eosinophil ratio was significantly high in patients with Kimura's disease compared with control. ($p < 0.05$)

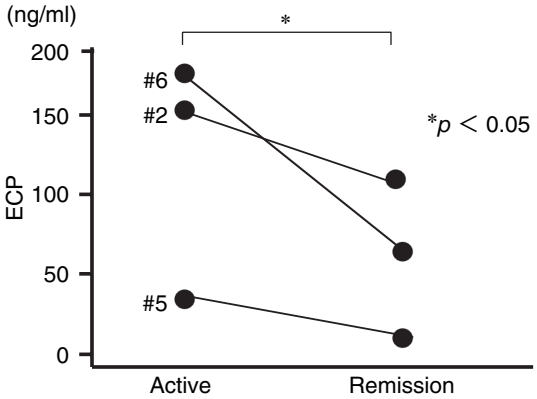

Fig. 4 The serum concentrations of ECP in 3 patients with Kimura's disease (patient #2, #5 and #6) in the active stage and the remission stage. The serum ECP levels were elevated in the active stage of Kimura's disease and were significantly decreased in the remission stage.

say (ELISA) kit (human GM-CSF assay kit; Genzyme, Denmark, IL-4 assay kit; R&D Systems, Inc., USA, human IL-5 assay kit; R&D Systems, Inc., USA).

STATISTICAL EVALUATION

Wilcoxon's signed-rank test, Mann-Whitney *U* test and linear correlation analysis were used.

RESULTS

CONCENTRATION OF ECP

Figure 1 shows the serum ECP values. The concentration of ECP in sera from patients with Kimura's disease was significantly higher than that in the control group ($p < 0.05$). Figure 2 shows that the serum ECP level was not significantly correlated with the number of eosinophils. Figure 3 shows that the ratio of serum concentration of ECP/the number of eosinophils was significantly higher than those of control. Figure 4 shows the serum concentration of ECP in 3 patients in the presence of eosinophilia and after the disappearance of eosinophilia.

IMMUNOHISTOCHEMICAL FINDINGS

In interfollicular areas, most infiltrated eosinophils were positive for EG2 antibody and the mean percentage of EG2-positive eosinophils was 75.7% (64.0–94.0%) (Table 1). In 2 cases of high levels of the ECP concentration, lymph node structure was well-preserved; marked follicular hyperplasia with large germinal centers and infiltration of eosinophils in interfollicular areas and marginal sinuses were observed, and infiltrated eosinophils were almost EG-2 positive (Figs. 5–7).

CONCENTRATION OF GM-CSF, IL-4, AND IL-5

The concentrations of GM-CSF were below the detectable levels in all sera examined. IL-4 and IL-5 were detectable in the sera from one out of 13 sera from 8 patients and these levels were within normal ranges.

DISCUSSION

Kimura's disease is an eosinophilic inflammatory disorder characterized by marked eosinophilia, an elevated titer of serum IgE, and recurrent subcutaneous inflammatory nodules. These subcutaneous lesions, often affecting the lymph nodes, characteristically display fibroinflammatory pseudotumors consisting of follicular lymphoid hyperplasia with prominent germinal centers, as well as interstitial fibroplasia with marked eosinophilic infiltration. It has been stated that Kimura's disease may be associated with allergy, endocrinic disorders, autoimmune diseases, parasites, viral infections and inflammation, although its etiology is not well-understood. Recent studies suggest, based on findings such as elevated serum IgE, an increased number of eosinophils in peripheral blood and in lesions, and an increased number of mast cells, that eosinophils may mainly be responsible for Kimura's disease.[1-5]

Recently, the role of the eosinophils in allergic diseases has been suggested by a number of studies.[6] Eosinophils contain several highly cytotoxic proteins, such as ECP, eosinophil peroxidase, eosinophil protein X, and major basic protein. ECP has been demonstrated to cause damage to airway epithelium resembling the histopathologic findings in asthma,[7,8] and *in vitro* study has shown that ECP acts upon mast cells and releases histamine.[8-11] To clarify the role of eosinophils in the pathogenesis of Kimura's disease, the concentrations of ECP in sera from patients with Kimura's disease were measured and the EG-2 positivity of eosinophils in the lesion was investigated immunohistochemically. Serum ECP levels were elevated in the active stage of Kimura's disease

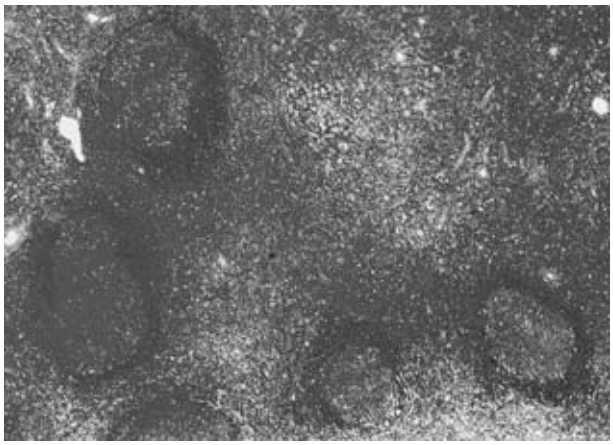

Fig. 5 A biopsy specimen from a right parotid of patient #4 showed characteristic proliferative lymph follicles with proliferation of blood vessels and infiltration of plasma cells and increased eosinophils partially forming eosinophilic abscesses. (HE, original magnification ×100)

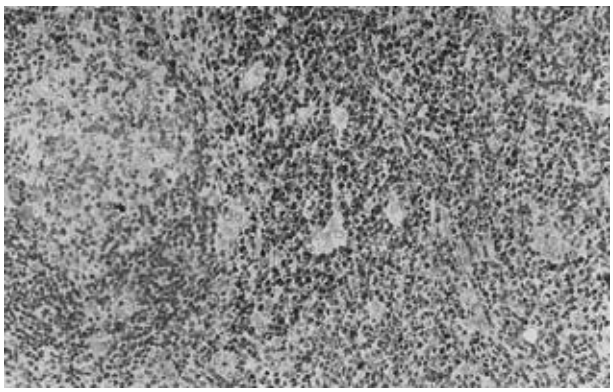

Fig. 6 A biopsy specimen from the right parotid of patient #4 showed the interfollicular area occupied with densely packed EG-1 positive eosinophils. (LF: secondary lymphoid follicle, counterstained with hematoxylin, original magnification ×250)

compared with those of healthy controls. As shown in Figures 2–4, the ECP levels were not significantly correlated with the number of eosinophils, and the ratio of ECP/eosinophils in patients with Kimura's disease was significantly higher than those of control. These findings suggested that ECP was not merely reflected with the number of the eosinophils, and surely released from activated eosinophils. Although ECP is probably important for the pathogenesis of Kimura's disease, there have been few case reports on the subject.[12] This is the first report to demonstrate the elevation of ECP in the sera from patients with Kimura's disease.

In the interfollicular areas, most infiltrated eosinophils were positive for EG-2 antibody; the mean percentage of EG2-positive eosinophils was 75.7%. These findings indicated that the infiltrated eosinophils of patients with Kimura's disease were activated. We speculated that the increase in the number of eosinophils may be caused by an increase in the production of eosinophils, the prolongation of their life span, or an increase in chemotaxis. Among the various hematopoietic factors, IL-4, IL-5, and GM-CSF stimulate eosinophil production and activate these cells.[4,5] To investigate the role of these cytokines in inductive eosinophilopoiesis and the activation of eosinophils in Kimura's disease, the serum concentration of IL-4, IL-5, and GM-CSF were measured. Contrary to our expectations, the serum concentrations of these cytokines were not elevated compared with those of healthy controls. The number of IL-4, IL-5, eotaxin and RANTES-expressing mast cells and T cells were increased in the lesions,[13] and IL-5 was produced from the site of a granuloma and lymph nodes of patients with Kimura's disease after stimulation with candida antigen.[4] Although the serum levels of GM-CSF and TNF were elevated and correlated with disease activity in a patient with Kimura's disease,[14] serum concentrations of IL-5, GM-CSF, and IL-3 were not detected in almost half of patients with various eosinophilia including Kimura's disease.[15] In asthma patients, a significant positive correlation was found between serum levels of ECP and IL-5, however, serum IL-5 levels were not high enough to evaluate the disease activity.[16] Sensitivity is the most concerning problem in the measurement system of IL-5. Although IL-5 is one of the factors that activate eosinophils in both local sites and peripheral blood, the measurement of serum IL-5 is not useful for monitoring Kimura's disease.

Based on these findings, we speculated that the in-

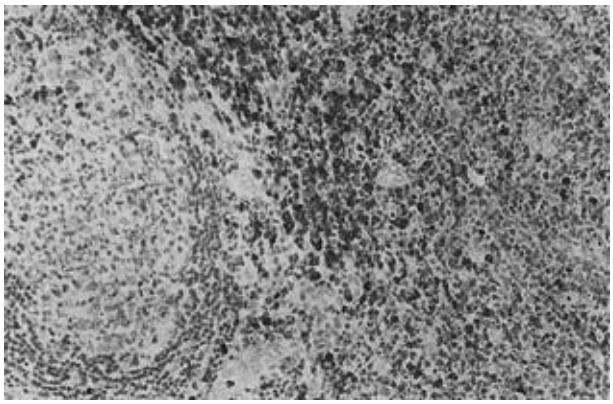

Fig. 7 A biopsy specimen from the right parotid of patient #4 showed the interfollicular area occupied with densely packed EG-2 positive eosinophils. (LF: secondary lymphoid follicle, counterstained with hematoxylin, original magnification ×250)

filtrated eosinophils of patients with Kimura's disease were activated and ECP may be used as an additional parameter of disease activity.

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