Periodic Angioedema with Eosinophilia: Increased Serum Level of Interleukin 5

Yoshio Okubo, Etsuro Sato, Mahboob Hossain, Teruo Ota, Sawako Yoshikawa and Morie Sekiguchi

A 48-year-old man developed episodic non pitting edema and eosinophilia. Symptoms were alleviated promptly when treated with prednisolone. However, major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) levels in the serum as well as the total number of eosinophils remained high. During periods of attack serum levels of interleukin-5 (IL-5) were elevated, but the levels were lowered following treatment, suggesting that IL-5 is involved in periodic angioedema with eosinophilia.

Introduction

Hypereosinophilic syndrome (HES) is a rare condition characterized by excessive numbers of circulating eosinophils with altered morphology and density (1). HES has been reported in patients covering a wide range of ages from 5 to 80 years. Untreated patients have a poor prognosis (2). Some variant forms of HES with angioedema have been reported to show a milder, longer term course without interfering with vital organ function (3-4). The cause of periodic angioedema with eosinophilia is unknown. Eosinophils play an important role in allergic and parasitic diseases and in other inflammatory disorders, probably through the release of toxic proteins into tissues (5).

Here, we report a patient with episodic angioedema and eosinophilia. A pathophysiological study in which the levels of eosinophil granule proteins in the patient’s serum were measured. We examined the serum levels of interleukin-5 (IL-5) during episodes of the illness and performed neutralization experiments on the sera using monoclonal antibodies against various cytokines.

Case Report

A 48-year-old man was admitted to Shinshu University Hospital in May 1989 with periodic systemic edema, skin tightness of both arms and legs, pruritic eruptions on the chest and neck, and marked eosinophilia. Until October 1988, when systemic edema, eruptions on the chest, neck, arm and face, myalgia and pruritus appeared, he had been healthy. He noticed these symptoms recurring approximately every 3 weeks. Symptoms lasted from 7 to 15 days. There was no history of bronchial asthma, food or drug allergy, or any other allergic disorders. The patient had never travelled abroad.

On admission in May 1989, during a period of recurrent edema, pruritus and myalgia, we examined leukocyte and eosinophil cell numbers, body weight and urine volume. The total leukocyte count at that time showed 12.1x10^9/l with 60% eosinophils, hemoglobin 16.1 g/dl and ESR 1 mm/h. No ova or parasites were detected in repeated stool examinations. Other peripheral blood tests were performed with normal or negative results except for IgE (1,312 IU/ml), RA (rheumatoid arthritis) test, and histamine (28 ng/ml/ normal <5 ng/ml). He had no symptoms of RA. High levels of histamine were found in additional test. Analysis of lymphocytes using flow cytometry (EPICS V: Coulter, Fl) showed a normal T cell count, normal percentages of helper and suppressor T cells, a normal helper/ suppressor T cell ratio and normal B cell count. Microscopically, 60% of the peripheral blood leukocytes were mature eosinophils, 7% of eosinophils showed 3 or more nuclear segments. Eosinophils with some vacuoles, a small number of specific granules in the cytoplasm which were reduced in size were observed by electron microscopy (data not shown). Hypodense eosinophils with these characteristics have been previously reported (6). Bone marrow aspiration was hypercellular with eosinophils similar to those in the peripheral blood or typical blood eosinophil precursors, but with no blast cells. There were no abnormalities on chest roentgenograms,
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pulmonary function test, ECG or echocardiogram. A skin specimen biopsy showed dermal edema, perivascular lymphocytic infiltration and diffuse eosinophilic infiltration. There was no evidence of vasculitis, granuloma formation, atrophy of sweat or sebaceous glands.

From July 1989 to August 1994, when the clinical symptoms reappeared the patient received oral steroid (prednisolone 15 mg/day) for 3 days. This therapy has helped to diminish the symptoms.

**Measurement of major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN)**

Peripheral blood specimens before and after treatment, and during some periods of periodic angioedema were drawn, which were left at room temperature for 45 min. Serum was obtained by centrifugation at 350 × g for 10 min at 15°C. MBP (7), ECP and EDN (8) were measured by RIA.

**Measurement of IL-5**

Serum specimens from the patient were assayed for IL-5 by immunoenzymometric assay: two monoclonal antibodies, JES1-39D10 as the coating antibody, and JES1-5A10 derived with hapten nitroiodophenyl as the detecting antibody, in conjunction with L-cell derived recombinant IL-5 as the standard, were used to measure IL-5. This assay has been used to quantify IL-5 in T-cell clone supernatants (9) in addition to mitogen-activated peripheral blood mononuclear cell supernatants (10). Serum specimens were stored at −20°C.

**Eosinophil survival assay**

Two hundred μl of purified human eosinophils (2.5×10⁴/well) in a flat bottom tissue culture plate (Falcon #3072) were incubated with patient’s serum or rhIL-5 (100 pg/ml or 0 pg/ml) for 4 days at 37°C, 5% CO₂ in a humidified atmosphere. After 4 days, 120 μl of supernatant was removed from each well, and 10 μl of fluorescein diacetate (11) was added to the cell suspension. After 15 min at room temperature, 10 μl of propidium iodide (12) was added. Then, the numbers of viable cells and dead cells were counted using a fluorescent microscope. Eosinophil survival was calculated as follows: percent of eosinophil survival = (viable cells)/(viable cells + dead cells)×100%. All experiments were performed in duplicate.

**Neutralization experiment**

Monoclonal antibodies (mAbs) against recombinant human interleukin-3 (rhIL-3), rhIL-5, recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF), recombinant human interferon-γ (rhIFN-γ, Genzyme Co., Boston, USA) and polyclonal antibody against recombinant human tumor necrosis factor alpha (rhTNF-α, Genzyme Co.) were used for neutralization. Serum specimens from the patient were each reacted with mAb or combinations of mAb for 1 hour at room temperature. Then the sera (10%, v/v) were cultured with freshly isolated eosinophils as previously described (13). These antibodies inhibited eosinophil survival by the corresponding cytokine in a dose-dependent manner in preliminary studies. Each 10 μg of anti-IL-5, anti-IL-3, and anti-GM-CSF mAbs completely neutralized rhIL-5 (300 pg), rhIL-3 (200 pg), and rhGM-CSF (20 pg), respectively. Anti-hIFN-γ mAb (2 μg) and anti-hTNF-α polyclonal antibody (5 μl) completely neutralized 800 U and 500 U of each cytokine, respectively. Each antibody of cytokine was specific for its respective cytokine.

**Statistical analysis**

All results are expressed as mean±SE. Significance was analyzed by paired Student’s t-test.

**Results**

The patient was admitted during a period of recurrent edema, pruritus, skin tightness and myalgia. We examined leukocyte and eosinophil cell numbers, body weight and urine volume. Figure 1 shows that leukocyte and eosinophil cell totals were minimal prior to the attack. During the attack, leukocytes and eosinophils increased, parallel to the gain in body weight. Body weight gradually decreased spontaneously with increased urine volume. Prednisolone (15 mg/day) was administered symptomatically for 3 days following the patient’s admission for treatment in May 1989. Symptoms, such as itching and...
Table 1. The Profile of Eosinophil Cell Count and Eosinophil Derived Proteins in Clinical Course

<table>
<thead>
<tr>
<th>Date</th>
<th>Eos (×10^3)</th>
<th>MBP (ng/ml)</th>
<th>ECP (ng/ml)</th>
<th>EDN (ng/ml)</th>
<th>Attack</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/17/’90</td>
<td>7,102</td>
<td>1,662</td>
<td>21</td>
<td>475</td>
<td>11/12-27</td>
<td>11/13-15 (15 mg ×3 days)</td>
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<tr>
<td>10/24/’90</td>
<td>696</td>
<td>1,107</td>
<td>14</td>
<td>308</td>
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<td></td>
</tr>
<tr>
<td>10/31/’90</td>
<td>1,690</td>
<td>1,323</td>
<td>48</td>
<td>195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/7/’90</td>
<td>1,136</td>
<td>1,620</td>
<td>87</td>
<td>199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/14/’90</td>
<td>2,438</td>
<td>1,909</td>
<td>385</td>
<td>753</td>
<td>11/12-27</td>
<td>11/13-15 (15 mg ×3 days)</td>
</tr>
<tr>
<td>11/21/’90</td>
<td>5,896</td>
<td>4,490</td>
<td>120</td>
<td>378</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/28/’90</td>
<td>4,880</td>
<td>2,677</td>
<td>150</td>
<td>550</td>
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<td></td>
</tr>
<tr>
<td>12/6/’90</td>
<td>1,972</td>
<td>1,077</td>
<td>43</td>
<td>268</td>
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<tr>
<td>12/12/’90</td>
<td>2,090</td>
<td>3,229</td>
<td>166</td>
<td>333</td>
<td>12/11-19</td>
<td>12/13-15 (15 mg ×3 days)</td>
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<tr>
<td>12/19/’90</td>
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<td>2,934</td>
<td>49</td>
<td>212</td>
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<td></td>
</tr>
<tr>
<td>12/26/’90</td>
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<td>1,405</td>
<td>52</td>
<td>369</td>
<td></td>
<td></td>
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<tr>
<td>1/9/’91</td>
<td>2,912</td>
<td>125</td>
<td>38</td>
<td>108</td>
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</table>

159±43 (N=20) 17±11 (N=50) 16±6 (N=20)

Eosinophil cell count, and amounts of major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) during periods of periodic angioedema. MBP, ECP and EDN were measured by RIA.

edema, rapidly disappeared. This pattern of symptoms recurred approximately every three weeks. However, the degree of pruritus became slightly less intense and myalgia disappeared after October 1989. We also examined leukocyte and eosinophil cell numbers, MBP, ECP, and EDN levels during some periods of recurrent edema and eosinophilia. Table 1 shows that high eosinophil cell numbers as well as high levels of MBP, ECP and EDN remained even when the symptoms disappeared after treatment.

Neutralization of patient’s serum

Figure 2 shows that the patient’s sera during some periods contained considerable eosinophil survival activity (70–94%). Eosinophil survival of normal sera was 12.8±5.6% (N=5). Eosinophil survival at 100 pg/ml (positive control) and 0 pg/ml (negative control) of rhIL-5 was 92% and 7%, respectively. Figure 3 shows a representative test of the patient’s serum for eosinophil survival (Dec. 12, 1990), significant inhibition occurred in the presence of anti-IL-5 mAb (P<0.01). Any combination of anti-IL-5 and other mAbs or polyclonal antibody inhibited eosinophil survival in the patient’s serum. Similar results were obtained in different samples (Oct. 24, Nov. 7, Nov. 14, Nov. 21, Nov. 28, 1990 and Jan. 9, 1991). Anti-GM-CSF and anti-IFN-γ mAbs showed a slight, insignificant tendency to inhibit eosinophil survival. Combinations of all monoclonal antibodies produced significant inhibition of eosinophil survival (P<0.05), however, alone anti-IL-3 mAb and anti-TNF-α had no effect.

Measurement of IL-5

We examined the relationship between eosinophil cell number and the serum levels of IL-5 during various periods of the syndrome. Table 2 shows that serum levels of IL-5 dropped immediately after treatment, but numbers of eosinophils and...
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Leukocyte, eosinophil cell count, and serum level of IL-5 were measured during periods of periodic angioedema. IL-5 was measured by immunoenzymic assay.

leukocytes were not consistently decreased immediately after treatment.

**Discussion**

The term hypereosinophilic syndrome (HES) was introduced by Hardy and Anderson in 1968 (14) to describe patients with peripheral blood eosinophilia for which no cause could be found. Chusid et al (15) defined the syndrome more precisely restricting the diagnosis to patients with 1) persistent eosinophilia (>1.5x10^9/l), 2) a lack of evidence for any known cause, and 3) evidence for multi-organ disease. The present patient with eosinophilia and periodic angioedema showed no evidence of parasitic, allergic or other disease which could cause eosinophilia. Periodic angioedema in this patient appeared approximately every 3 weeks and persisted for up to 15 days. The mechanism of periodic angioedema is not clear. The patient gained about 8% of his normal weight. Interestingly, the total cell number of eosinophils increased during an attack regardless of prednisolone treatment. During an attack there was no significant change in hematocrit or RBC. From these data, it can be suggested that the increased number of eosinophils was not due to hemoconcentration. The levels of MBP, ECP and EDN in the patient’s serum were always high during an attack and remained high even after treatment. The mechanism of periodic angioedema is not clear. The patient’s serum showed periodic increases which dropped after treatment, thus IL-5 increased in the serum in accordance with periodic eosinophilia. Further, a relationship was found between the level of IL-5 and the prednisolone treatment. However, the eosinophil cell number was not significantly reduced by the treatment. Possibly the dose of prednisolone was not sufficient or other eosinopoietic factor(s) were involved in the periodic angioedema. Taken collectively, these data suggest that IL-5 is involved in periodic angioedema with eosinophilia.

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**References**