Scoring systems and cell culture studies in patients with hypereosinophilic syndrome

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Abstract

Five patients with hypereosinophilic syndrome (HES) were evaluated by hematologic and clinical scoring systems and cell culture studies. An increased number of CFU-C, and increased number and proportion of eosinophil colonies were observed in bone marrow specimens from 3 patients who had low scores. In a patient with HES who had a high score, an increase of eosinophil clusters in the bone marrow, marked increases of CFU-C and eosinophil colonies in the peripheral blood, and of eosinophil colony-stimulating activity in the plasma were observed. These results suggest that cell culture studies as well as the scoring systems seem useful in evaluating patients with HES.

Key words: Scoring system, Cell culture, Hypereosinophilic syndrome

Five patients with hypereosinophilic syndrome (HES) were evaluated by the scoring systems devised by Flaum et al [1] and Schooley et al [2]. One of these patients died of progressive organ system infiltration similar to eosinophilic leukemia. A heterogeneity of cultural findings was reported in patients with HES [3, 4]. Instituting a method to evaluate patients early-stage HES to initiate appropriate therapy could be clinically useful. This paper reports cell culture studies that could be useful in predicting the clinical course and distinguishing the aggressive form of HES or eosinophilic leukemia from mild form of HES.

Materials and methods

Hypereosinophilia patients

The criteria for the diagnosis of HES as outlined by Chusid et al [5] are: (1) a persistent eosinophilia of 1.5×10^9 eosinophils/l for longer than six months; (2) lack of evidence for any other cause of eosinophilia; and, (3) evidence of organ system involvement.

Five patients were selected who were retrospectively considered to have HES, as defined by Chusid et al [5]. The clinical findings are presented in Table I. None of the patients had symptoms to suggest allergy and no clinical or serological evidence of a parasitic infection. There was no evidence of cutaneous or connective tissue disease. Prednisone is the useful drug utilized if patients manifest significant symptomatology. Patient 5 was treated with cytotoxic agents, but died of congestive heart failure due to progressive organ infiltration similar to that observed in leukemia [6, 7].

Case report (Table I. patient 5)

A 16-month-old boy was admitted on 7 October 1980 with a history of low grade fever. Physical examination was unremarkable except for swelling of both hands and feet. Laboratory tests showed: hemoglobin 9.7 g/dl; white blood cell count 168.9×10^9/l with 1% segmented neutrophil, 11% lymphocytes and 88% eosinophils (86% segmented, 2% band forms), many of eosinophils were vacuolated and degranulated; platelet count 183.0×10^9/l; a slightly low leukocyte alkaline phosphatase count; slightly increased vitamine B12; and no chromosomal abnormalities. A bone marrow aspirates was markedly hypercellular with reduced but normoblastic

Table I . Clinical data on 5 HES patients studied

Patient	Sex	Age (yr)	WBC (× 109/1)	Eosinophils (× 109/1)	Organ involvement	Clinical features
1	M	47	19.0	8.4	Skin	Developed colon cancer. died
2	M	25	25.4	14.6	Liver. spleen	Responded to steroids Remains well on no treatment
3	M	4	9.0	2.5	Brain. peripheral nerve	symptoms persist
4	M	10	27.0	14.3	Gastrointestinal	Responded to steroids Remains well on no treatment
5	M	1	168.9	153.6	Heart. peripheral nerve lung. liver, skin. renal	Unresponsive to chemotherapy died with a heart failure

erythropoiesis, 4.7% myelocytes, 3.3% metamyelocytes, 0.7% basophils, and 60.9% eosinophils (35% segmented eosinophils, 6.3% band forms, 11.3% metamyelocytes, and 8.3% myelocytes).

The patient was treated with prednisone without effect. Then he was treated with cytosine arabinoside and vincristine. There was a transient hematological response to chemotherapy and hydroxyurea. Eventually he died of congestive heart failure with progressive organ infiltration. Autopsy findings revealed endomyocardial fibrosis and mural thrombus.

Hematologic and clinical scoring systems

The hematologic and clinical grading scores were determined by the grading system of Flaum et al [I] and Schooley et al [2]. The hematologic scoring system was based on peripheral blood and bone marrow findings, cytogenetics, B12 levels, and the clinical scoring system was based on clinical features involving the 8 organ systems commonly affected by the illness. (Table II)

Table II . Hematologic and clinical scoring systems

Hematologic scoring system
Peripheral blood
Anemia 2, Abnormal red blood cell morphology 1, Increased platelet 1,
Decreased platelet 2, Myeloid dyspoiesis of hypersegmentation 1, Basophilia 2,
Immature WBCs 1-2
Bone marrow
Hypercellularity 1-2, Decreased megakaryocyte 1, Myelofibrosis 2, Myeloid dyspoiesis 2,
Basophilia 2, Myeloblast-progranulocytes (>5%)2

Abnormal cytogenetics 2, Increased B12 1, Abnormal LAP 1

Clinical grading system
Organ system involovement
Cardiovascular 2-5, Nervous system 2-5, Lungs 2-3, Hepatosplenic 2, Gastrointestinal 1,
Kidneys 3, Muscles 1, Cutaneous -1-2

Clonal cell cultures

Bone marrow or peripheral blood samples were aspirated into a syringe containing preservative-free heparine, layered on Ficoll-Hypaque, and centrifuged at 1500 rpm for 25 minutes. Mononuclear cells were harvested and washed twice in alpha medium (Flow Laboratories, North Ryde, Australia).

Cell culture for colony forming unit in culture (CFU-C) were carried out, using a modification of the technique described by Iscove et al [8]. Briefly, one ml of culture mixture containing 1×10^5 non adherent cells, alpha medium, 20% fetal calf serum (FCS)(Flow), 20% phytohaemagglutinin-leucocyte conditioned medium (PHA-LCM), and 0.96% methylcellulose was plated in a 35 mm culture dish. Triplicate culture were incubated at 37℃ in a humidified atmosphere of 10% CO_2. On day 14, colonies of 40 or more cells and clusters of fewer cells were counted. Granulocyte-macrophage colonies and eosinophil colonies were identified in vitro by their morphological appearance under inverted microscopy. Granulocyte-macrophage colonies had a diffuse polymorphic appearance, and eosinophil colonies appeared as tight collections of small round cells under inverted microscopy. Colony morphology was determined at intervals in whole dishes, after drying of the methylcellulose and staining eosinophil colony cells with Biebrich-scarlet stainig. The number of total colonies (granulocyte-macrophage colonies plus eosinophil colonies) was counted as CFU-C.

The plasma from the patients, as well as a control AB plasma, was tested at 5%, in place of FCS as a source of colony-stimulating activity (CSA) for normal human bone marrow.

Results

Hematologic and clinical scores

The hematologic and clinical scores of the 5 patients with HES are summarized in Table Ⅲ.

Table Ⅲ. Hematologic and clinical scoring of 5 patients with hypereosinophilic systems

Patient	Hemaologic score	Clinical score
1	1	2
2	2	2
3	1	5
4	1	1
5	12	16

Four patients (patients 1,2,3, and 4) had low hematologic and clinical scores, and either did not require therapy or had responded to prednisone therapy. One patient (patient 5), who had significantly higher hematologic and clinical scores, died of progressive organ system infiltration despite prednisone and cytotoxic therapy.

Cell Culture

The number of CFU-C and eosinophil colonies in the specimens of bone marrow from the patients with HES are shown in Fig. 1. An increased number of CFU-C, and increased number and proportion of eosinophil colonies were observed in the bone marrow from 3 patients (Fig.1; 2, 3, and 4) who had low hematologic and clinical scores. In case 2 (Fig. 1; 2), the total number and the proportion of eosinophilic colonies decreased after treatment with prednisone. In patient 5 who had high hematologic and clinical scores, the number of CFU-C and eosinophil colonies were reduces, while a marked increase of eosinophil clusters was seen. The ratio of eosinophil colonies to clusters was 1:1 in cases 1, 2, 3 , and 4, while this ratio was 1:14 in case 5.

Fig. 2 shows the number of CFU-C and eosinophil colonies in peripheral blood. The number of CFU-C were within the range of normal control in cases 1, 2, 3, and 4, while a marked increase of CFU-C and eosinophil colonies was seen in case 5 (Fig. 2; 5).

In case 2 (Fig.3; 2), EO-CSA was increased when the patient was first tested and decreased after treatment with prednisone. In case 5 (Fig.3; 5), the eosinophil CSA (EO-CSA) was markedly increased, while the granulocyte-macrophage CSA (GM-CSA) increased little compared to the control.

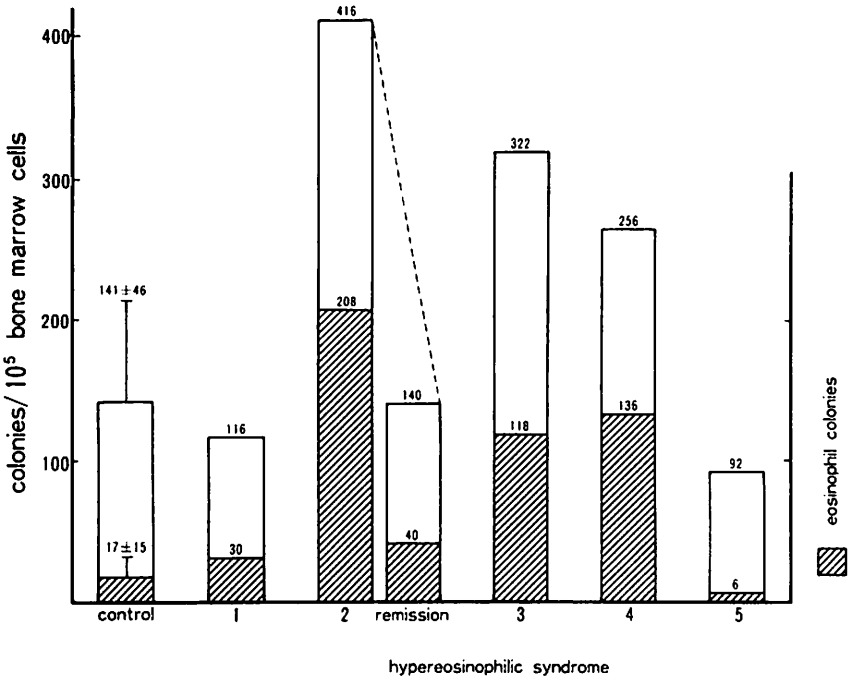

Fig. 1 CFU-C in bone marrow from patients with hypereosinophilic syndrome
Data of control group (n=23) represent mean SD. Data of patients represent mean of triplicate plates.

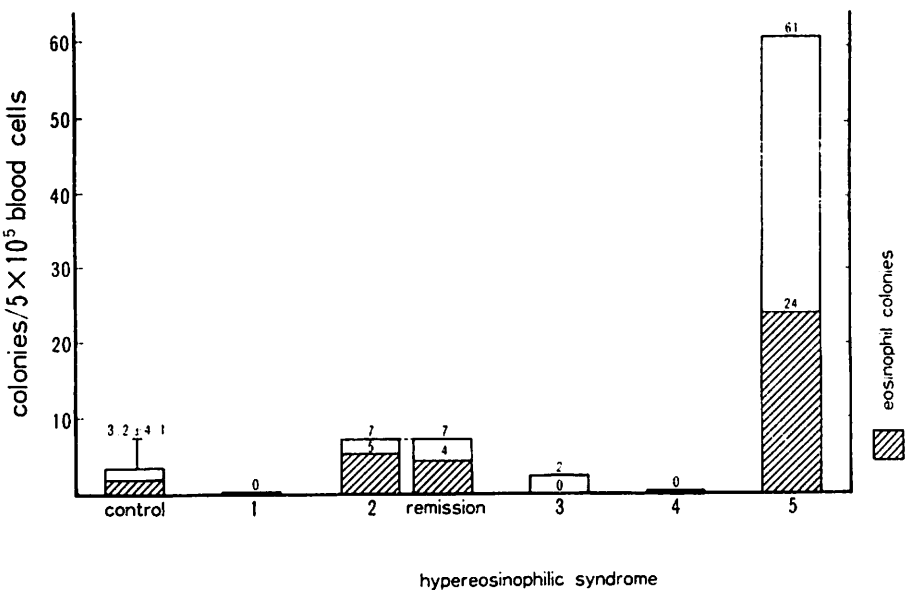

Fig. 2 CFU-C in perpheral blood from patients with hypereosinophilic syndrome
Data of control group (n=23) represent mean SD. Data of patients represent mean of triplicate plates.

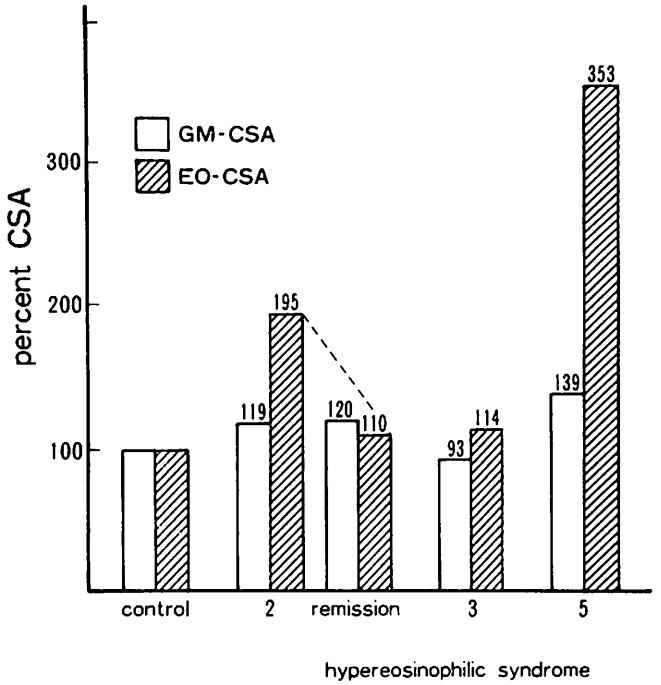

Fig. 3 Colony-stimulating activity in the plasma from patients with hypereosinophilic syndrome
Data of patients represent mean of triplicate plates. CSA=coony-stimulating activity; GM-CSA=granulocyte-macrophage CSA; EO-CSA=eosinophil CSA

Discussion

This paper describes the hematologic and clinical scoring systems and cell culture findings in 5 patients with HES.

Four patients, who had low hematologic and clinical scores, either did not require therapy or had responded to prednisone therapy. One patient, who had high scores, died of progressive organ infiltration similar to eosinophilic leukemia. The hematologic and clinical grading systems seem useful in predicting the clinical course as others have previously reported [1, 2].

An increased number of CFU-C, and increased number and proportion of eosinophil colonies were observed in the bone marrow from 3 patients who had low scores and normal in the fourth.

Bone marrow CFU-C were not reduced in HES, although the number of patients were small [4, 9]. In 20 patients with reactive eosinophilia, mainly due to helminthic disease, Kern and Dietrich [10] found a moderate reduction of bone marrow CFU-C. In two patients with eosinophilic leukemia, marrow CFU-C were within normal range [7, 9]. They reported that culture of marrow derived progenitor cells may be less useful for the diagnosis of eosinophilic leukemia, since the number of colonies, and the proportion which were eosinophilic were similar to those found in patients with HES. In patient 5, a marked increase of eosinophil clusters was seen, while CFU-C number and eosinophil colonies were reduced. This in vitro growth pattern may support the malignant nature of progenitor cells in this patient, since cluster formation represents a proliferation pattern of leukemic cells [11,12]. Furthermore the marked increase of CFU-C and eosinophil colonies in this patient contrasts with the normal number of CFU-C found in patients with HES [3,4]. This growth pattern is similar to that seen in chronic granulocytic leukemia and in two patients with eosinophilic leukemia [6,7].

There is some correlation between the production of EO-CSA and the number of circulating eosinophils. As

shown in Figure 3, the plasma of patient 3 with the lowest number of eosinophils produce GM-CSA and EO-CSA very close to controls, while patient 2 (14.6 eosinophils) were two fold higher EO-CSA, and patient 5 (153.6 eosinophils) three and half fold higher. After treatment with prednisone (normal number of eosinophils), patient 2 give a normal EO-CSA.

The qustion remains unresolved whether the study of CFU-C and CFU-EO bone marrow growth patterns can discriminate between eosinophilic leukemia and the aggressive types of HES.

References

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要　　旨

Hypereosinophilic syndrome（HES;好酸球増多症候群）5名の患者を、従来の血液学的、臨床的スコアで評価するとともに、in vitroコロニー形成法にて細胞培養を行った。

スコアが低い3名のHES患者で、骨髄CFU-Cと好酸球コロニー比率の増加が認められた。スコアが高く、好酸球性白血病類似の経過を取ったHES患者で、骨髄好酸球クラスターの増加、末梢血CFU-Cと好酸球コロニー、血漿好酸球コロニー形成刺激因子の著増が認められた。

HES患者を評価するには、血液学的、臨床的スコアとともにin vitro細胞培養法が有用である可能性が示唆された。