T cell lymphoid aggregates in bone marrow in idiopathic hypereosinophilic syndrome

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Abstract

Idiopathic hypereosinophilic syndrome (HES) comprises a heterogeneous group of disorders characterised by prolonged eosinophilia with no obvious cause. A patient with longstanding HES is reported in whom unusual non-neoplastic peritrabecular lymphoblastic aggregates were present in the bone marrow, a hitherto undescribed association, as far as is known. An eosinophil colony stimulating activity was detected in the serum. The findings in this patient provide further evidence for an important role for eosinophil colony stimulating activity interleukin-5 mediated T lymphocyte control of eosinophil production in the pathogenesis of the HES.

(3 Clin Pathol 1993;46:955–958)

Idiopathic hypereosinophilic syndrome (HES) includes a heterogeneous group of disorders in which there is prolonged eosinophilia of undetectable cause and various organs affected. Interleukin 5 (IL-5), a product of activated T lymphocytes, is an important cytokine involved in the production of eosinophils. Increased IL-5 concentrations have been reported in patients with HES, prompting the suggestion that excessive quantities of IL-5 may account for the characteristic eosinophilia. We report a patient with longstanding HES in whom unusual, non-neoplastic peritrabecular T cell lymphoid aggregates were present in the bone marrow, an association, which, to our knowledge, has not been recorded before.

Case report

The patient was a 58 year old male spray painter. He originally came from Sicily and had been in Australia since 1958. He had been well until 1978, when, aged 44, he was admitted to hospital with a deep venous thrombosis. Hepatomegaly and spleen palpable 2 cm from the costal margin were noted. Blood examination showed a total leucocyte count of 10·1 × 10⁹/l, with a pronounced eosinophilia of 2·7 × 10⁹/l. A bone marrow aspirate indicated hypercellular particles, with increased numbers of eosinophils and eosinophil precursors. A marrow trephine biopsy was not carried out at that time. A liver biopsy specimen showed portal fibrosis with a dense infiltration of eosinophils in the portal tracts. No cause for the pronounced eosinophilia could be established, and HES was diagnosed.

The patient presented to the Royal Melbourne Hospital in May 1992 for the investigation of a heart murmur. Examination identified pityriasis versicolor of the skin, an enlarged liver, and a spleen palpable 3 cm below the costal margin. There was no lymphadenopathy. Cardiac investigation revealed mitral and tricuspid incompetence; a myocardial biopsy specimen showed histological features consistent with endocardial fibrosis. A skeletal survey indicated no abnormality.

Blood examination showed a haemoglobin value of 139 g/l, a platelet count of 124 × 10⁹/l, and leucocytes at 12·1 × 10⁹/l, with neutrophils 4·59 × 10⁹/l, monocytes 0·84 × 10⁹/l, and lymphocytes 1·93 × 10⁹/l. Pronounced eosinophilia was present, the eosinophils numbering 4·71 × 10⁹/l. Many of the eosinophils showed areas of cytoplasmic hypogranulation. Serum vitamin B₁₂ was greater than 1800 pg/ml and the serum IgE titre was normal. A bone marrow aspirate and a trephine biopsy specimen were taken.

Investigations aimed at establishing a cause for persistent eosinophilia were all negative. Antibodies to Schistosoma mansoni and Toxocara canis were not detected by enzyme immunoassay. No parasites were detected in

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Accepted for publication 22 April 1993
Methods
The haematological methods used were standard. Eosinophil colony stimulating activity in serum was assayed using normal human bone marrow cells cultured in agar for 14 days in a humidified atmosphere of 10% CO₂, as previously described. Serial dilutions of serum were used to stimulate cell growth. Cultures were then fixed and stained with Luxol Fast Blue and the number of cells counted in consecutive eosinophil clones using a light microscope. Clones were defined as greater than five cells and colonies as greater than 50 cells. To examine T cell receptor γ chain rearrangement a polymerase chain reaction based method was performed, as described by Trainor and colleagues.

Results
A bone marrow aspirate and a trephine biopsy specimen were taken from the posterior iliac crest. The aspirate showed moderately hypercellular particles and trails. Erythropoiesis was normocellular and normoblastic. Granulopoiesis was normal except for a substantial increase in eosinophils which constituted 15% of the total nucleated cells. All stages of eosinophil maturation were increased. Many of the eosinophils showed abnormal granulation, with failure to fill the cytoplasm with granules, and concentration of granules towards one pole of the cell. Megakaryocytes were normal in number and appearance. Lymphocytes (8-5%) and plasma cells (2%) were not increased.

The bone marrow trephine biopsy specimen comprised two cores, the larger measuring 10 x 2 mm. The myeloid:erythroid ratio was increased (about 5:1), with preservation of normal architecture. Granulopoiesis was hypercellular due to a substantial increase in eosinophils, in all stages of development. Erythropoiesis was normocellular, the megakaryocytes were normal in number and appearance, and the bony trabeculae were unremarkable.

Punctuating the marrow were three discrete, moderate-sized lymphoid aggregates, two of which were peritrabecular, almost completely surrounding bony trabeculae (figs 1A and B). The lymphocytes within the aggregates were small round mature cells, without clefting or nucleoli. An occasional macrophage was interspersed among the lymphoid cells. Dense collection of eosinophils cuffed the lymphoid collections (fig 2). There was a moderate increase in reticulin within the lymphoid aggregates which extended into adjacent areas.

Staining of the marrow sections with various immunohistochemical antibodies showed that almost all the lymphocytes in the aggregates were positive for T cell markers CD 45RO (UCHL-1) and CD 43 (leu 22). Only occasional lymphocytes were of B cell lineage (L26 or CD20 positive). The macrophages were immunopositive for CD68 (KP1). No S-100 protein positive giant cells were evident and staining for λ and κ light chains showed

the faeces on wet preparation, with formalin concentration, or using a trichrome stain. Treatment with prednisone was started. Follow up examination six months after the initial investigation showed no progression of the disease, with no increase in splenomegaly and no lymphadenopathy.
only background positivity. Cytogenetic studies on the marrow aspirate indicated a normal karyotype.

Eosinophil colony stimulating activity was detected in the serum. The size of 19 consecutive eosinophil clones was (mean (SD)) 30.5 (31.7) cells for cultures stimulated using a final serum dilution of 1 in 50. There were four eosinophil colonies (size 88 (15.5) cells). In cultures stimulated with serum at a final dilution of 1 in 250 the size of eosinophil clones was also increased (17.4 (11.5) cells) (n = 17) with six clones of more than 20 cells per clone. In comparison, in unstimulated control cultures the average size of eosinophil clones was 9.6 (4.5) cells (n = 24) with no clones attaining 20 cells in size. There was no detectable increase in the number or size of granulocyte-macrophage clones in stimulated cultures. An increase in both the number of eosinophil colonies and size of eosinophil clones, evidence of an eosinophil specific stimulatory activity present in serum, was demonstrated in cultures.

Gene rearrangement studies on peripheral blood showed no clonal rearrangement of the T cell receptor λ locus, consistent with a polyclonal expansion of T lymphocytes.

**Discussion**

The diagnostic criteria for the HES are: (1) persistent eosinophilia of greater than 1.5 × 10^9/L, (2) lack of evidence for other recognised causes for eosinophilia; and (3) organ disease. Our case fulfils all these criteria. There was persistent eosinophilia for at least 14 years, affecting spleen, liver, and heart, and all investigations to establish a cause for the eosinophilia were negative. The occurrence of deep vein thrombosis, skin lesions, abnormal eosinophil granulation, thrombocytopenia, and increased serum vitamin B12 are all well recognised in HES. The duration of illness in our patient was unusually prolonged. Mean duration of disease has been reported as 4-8 years, but the range is wide, ranging from one to 24 years.

There have been few detailed reports of the bone marrow findings in HES. In general, the marrow is reported as showing only increased eosinophils. In a study of 50 patients with HES increased cellularity, fibrosis, decreased megakaryocytes, basophilia, myeloid dyspoiesis, and eosinophilia with increased numbers of immature forms were reported in the marrow, but no mention was made of lymphoid aggregates.

The presence of lymphoid aggregates in the marrow of unusual peritrabecular distribution has, to our knowledge, not been reported before in patients with HES. The association might be incidental and the lymphoid aggregates might represent nodules that would be encountered in normal bone marrow trephine samples. This seems most unlikely, in view of the number of aggregates present in the biopsy specimen, their unusual peritrabecular distribution, and their almost exclusive composition of T lymphocytes.

The association of eosinophilia with lymphoproliferative disorders, such as lymphoblastic leukaemia and lymphoma is well recognised. Recently, three patients with HES in whom there was associated lymphomatoid papulosis were reported. Immunocytochemical studies of the cutaneous lesions in two of the patients suggested a mature T cell phenotype with a predominant population of CD4 positive cells. Kim and colleagues...
described a case of HES terminating as a T cell lymphoma in a 3 year old girl. There was disseminated T cell lymphoma affecting lymph nodes, liver, lung, and kidney.

In the patient reported here the lymphoid aggregates in the bone marrow might represent the development of a T cell lymphoma against the background of the longstanding HES. This seems unlikely for several reasons. Enlarged liver and spleen had been noted at the onset of the disease 14 years earlier, and the degree had not been progressive, nor was there evidence of the development of any other lymphoid proliferations, such as lymphadenopathy. Histologically, the findings in the lesion were not consistent with a malignant T cell lymphoma. Marrow cultures stimulated with phytohaemagglutinin failed to indicate any cytogenetic abnormality. In support of a polyclonal T cell expansion, no clonal rearrangement of the T cell receptor γ locus was detected. Furthermore, in the six months since the detection of the lymphoid aggregates in the marrow, no evidence of lymphoma has developed.

The mechanism whereby T cell proliferations induce eosinophilia is likely through cytokine production. Important eosinophilic regulating factors such as granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, and IL-5 are produced by activated T cells, and eosinophil stimulating activity has been detected in the serum of patients with HES. Although the factor responsible for this has not been fully characterised in all the studies reported, it probably represents IL-5. Similarly, in the present study, although other activities such as GM-CSF cannot be completely excluded, the eosinophil colony stimulating activity was probably IL-5 because of the absence of detectable activity for granulocyte-macrophage progenitor cells.

Raghavachar and colleagues established T lymphocyte clones from the blood of a patient with HES. One third of these clones preferentially stimulated pure eosinophil colonies when cocultured with normal bone marrow cells. Analysis of the T cell receptor β-chain rearrangement of the eosinophil colony stimulating factor producing clones showed a different rearrangement pattern for each clone, suggesting a reactive T cell lymphocytosis, mediating eosinophilia as the pathogenetic mechanism for HES.

We thank E Delvia for help with the agar cultures.

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J Clin Pathol 1993 46: 955-958
doi: 10.1136/jcp.46.10.955

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