

Hairy Cell Leukaemia - Diagnosis and Current Treatment

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Introduction

Hairy cell leukaemia is a chronic B cell disorder which runs an indolent but progressive course characterized by cytopenia, recurrent infections and splenomegaly¹⁻³. The hallmark of this disease is the presence of cells with filiform cytoplasmic surface projections in peripheral blood and/or bone marrow. In recent years the introduction of new chemotherapeutic agents has revolutionized the treatment and prognosis of this condition, Interferon alpha is useful in controlling the disease, while drugs affecting nucleotide metabolism offer the possibility of lasting remission.

Epidemiology

Hairy cell leukaemia accounts for 2% of all leukaemias with an overall incidence of 2 cases per million of the population¹. The median age at diagnosis is 52 years and more than half of the cases present in the fifth and sixth decade of life. Affected males outnumber females 6:1. Before the introduction of systemic therapy the median survival was 4 years although a few patients survived for as long as 25 years²⁻⁴. It remains to be seen how far new therapeutic regimens will alter the median survival in this disease.

Aetiology

The aetiology of hairy cell leukaemia is unknown⁵. Infection with Epstein Barr Virus (EBV), cigarette smoking, chronic anaemia, woodworking and framing, routine use of aspirin or tranquilizers and prior exposure to chemicals have all been suggested as possible predisposing factors; but no firm evidence has emerged to support any association with hairy cell leukaemia⁶. Chang et al. used a genomic EBV internal repeat probe and an oligonucleotide probe directed against the EBER1 gene, but were unable to demonstrate a link between EBV and hairy cell leukaemia⁷. Genetic factors may be involved in the aetiopathology, since hairy cell leukaemia has been reported in patient's first degree male relatives and to date at least 15 such cases have been described in the literature⁸.

Pathology

Hairy cells infiltrate bone marrow (Figure 1),

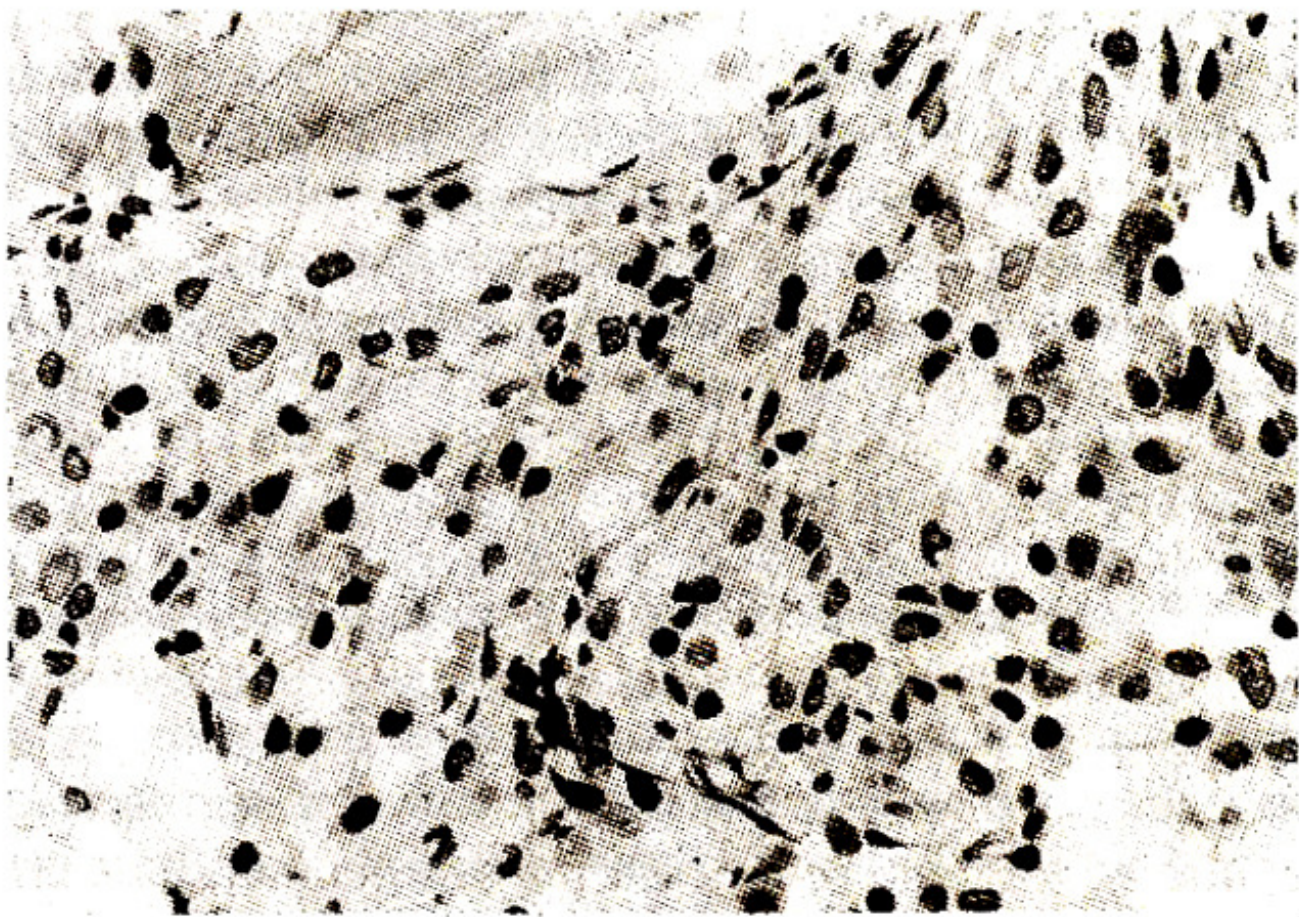


Figure 1. Bone marrow trephine biopsy, hairy cell leukaemia, showing a loose infiltrate of hairy cells with round and oval nuclei surrounded by wide intercellular spaces. Haematoxylin and eosin x 500.

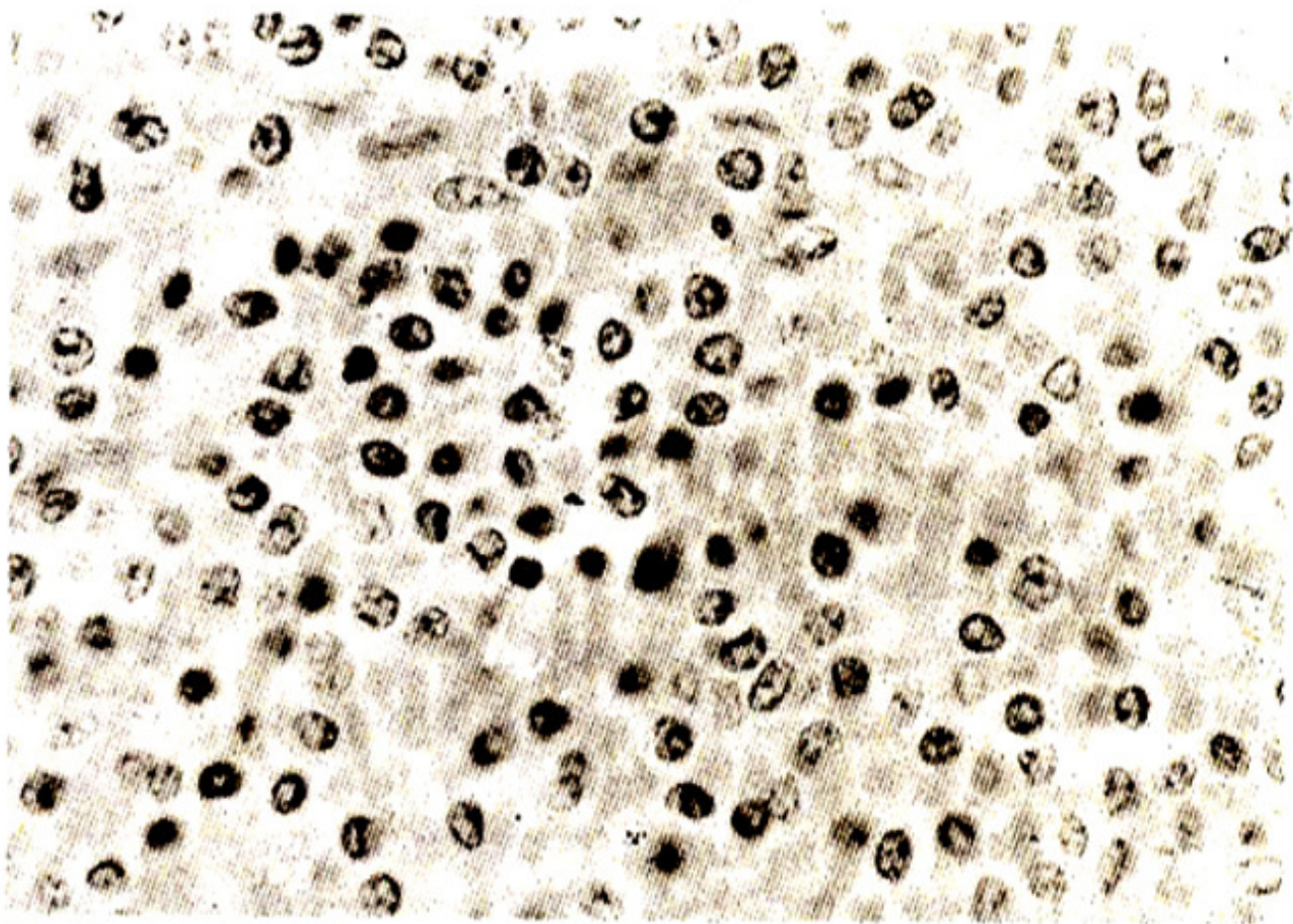


Figure 2. Spleen, hairy cell leukaemia, hairy cell infiltration of the red pulp gives the appearance of pseudosinuses. Haematoxylin and eosin x 500.

spleen (Figure 2), lymph nodes and skin. Cytopenia and organomegaly reflect the degree of organ involvement. The bone marrow shows diffuse infiltration by hairy cells, with increased fibroblastic activity and reticulin deposition; in consequence simple marrow aspiration results in a 'dry tap' in 40% of patients⁹. When marrow fragments are obtained smears typically show large mononuclear cells with abundant cytoplasm and delicate surface projections. Their nuclei are round, oval or indented with no visible nucleoli. Trephine biopsy of the marrow is advisable in all cases to support the diagnosis and to determine the response to treatment¹⁰. Histologically the marrow is replaced by a loose mononuclear cell infiltrate which may be patchy or diffuse¹⁰⁻¹³. Wide intercellular spaces give the appearance of clear zones between the abnormal cells. Connective tissue stains reveal fibrosis with increased reticulin deposition which typically has a dense mesh like appearance. Electron microscopy of hairy cells shows a characteristic abnormality known as a ribosome-lamella complex in 30-50% of cells. These structures consist of a sheath of parallel lamellae with rows of evenly spaced ribosomes in between them. The spleen shows, uniform smooth enlargement in 80% of cases and histologic examination shows hairy cell infiltration of the red pulp. White pulp is scanty and pseudosinuses are formed in the red pulp resulting in the appearance of so called red cell lakes¹⁴. Lymphadenopathy is rare in the early stage of the disease but when present is associated with a diffuse infiltrate of widely spaced hairy cells³.

Clinical Features

Most patients seek medical attention on account of non-specific symptoms of ill health such as weakness, weight loss, tiredness, lethargy and shortness of breath, infection of the consequences of

cytopenias⁵. Pyogenic infections with gram positive Cocci and gram negative bacilli are common in neutropenic patients, but infections with atypical mycobacteria and toxoplasmosis also occur frequently suggesting defective cell mediated immunity. Bone pain, vasculitic rash, arthritis, polyarteritis nodosa and systemic vasculitis may complicate the course of the disease at some stage but occasionally are present at the time of diagnosis. Splenomegaly is detectable by clinical examination in approximately 80% of patients; the spleen is more than 10 cm below the costal margin in about a quarter to one third of them^{2,5}. There is hepatomegaly in 30-40% of cases⁵. Lymphadenopathy was recently reported in 8 out of 27 new patients (30%) in whom computerized tomographic scans (CT scan) of the abdomen were performed at diagnosis and in 19 out of 50 patients (38%) scanned during the course of their disease, but clinically apparent lymph node enlargement is unusual. Lymphadenopathy is associated with significant splenomegaly, long standing disease and resistance to treatment¹⁵.

Laboratory Features

At presentation patients with hairy cell leukaemia as a rule show one or more cytopenias or even pancytopenia depending upon the extent of marrow infiltration and splenomegaly. The total white cell count may be low, normal, or high, but is rarely more than $20 \times 10^9/l$ ^{2,5}. Neutropenia and monocytopenia are usual^{9,16}. Hairy cells are found in the blood in up to two thirds of cases (Figure 3).

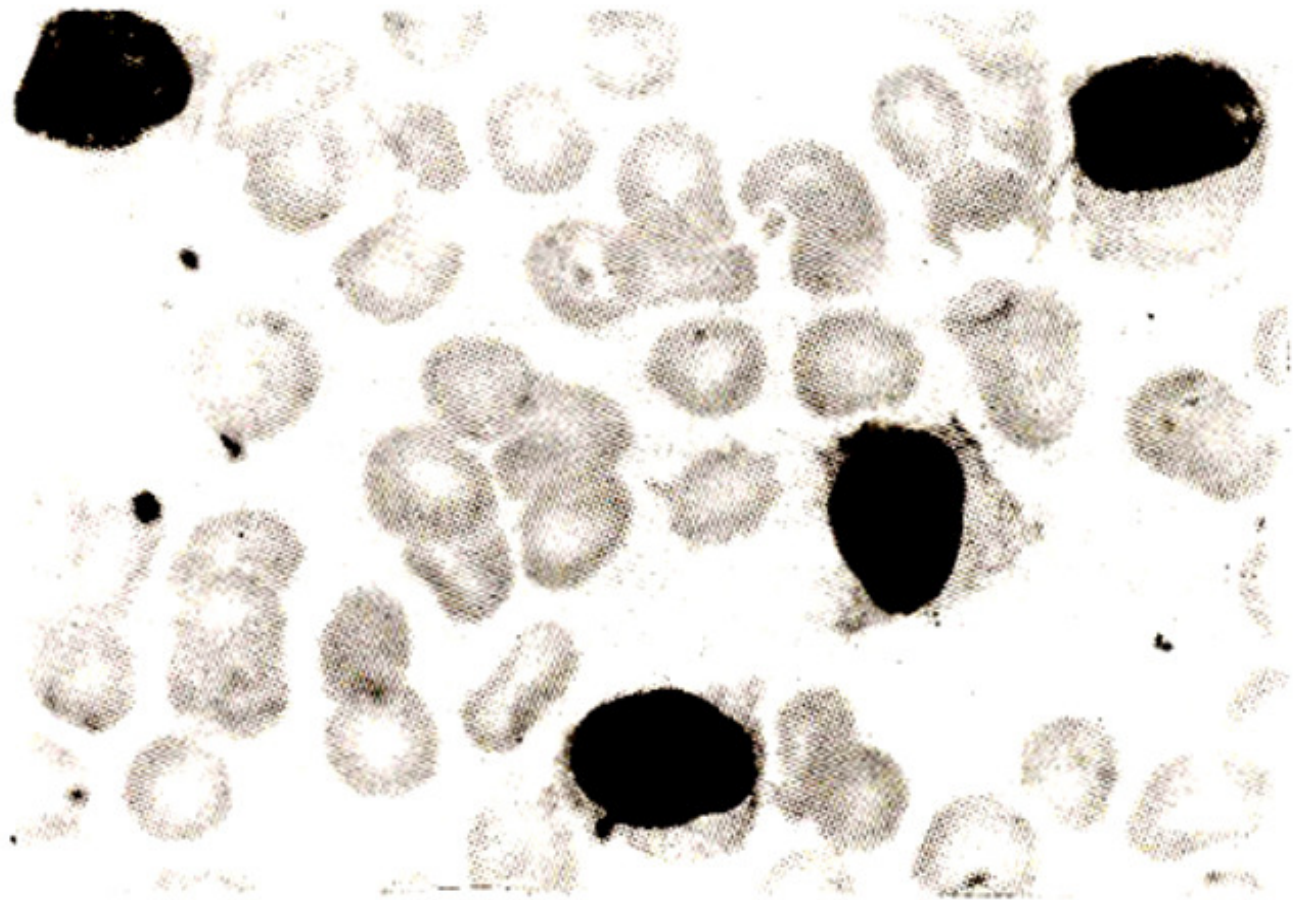


Figure 3. Peripheral blood film, hairy cell leukaemia, note the hairy cytoplasmic projections. May Grunwald and Geimsa x 1000.

A minority have few or no detectable hairy cells on blood film examination. Mild to moderate thrombocytopenia is found in 80% of cases, but symptoms due to severe thrombocytopenia are infrequent. Red cells are often mildly macrocytic and show anisocytosis⁹. Anaemia is usually the result of bone marrow infiltration and replacement by malignant cells^{2,5}. Hairy cells produce a factor or

factors, possibly tumour necrosis factor (TNF) which inhibits granulocyte/macrophage colony forming units (CPUGM) and erythroid burst forming units (BFU-E) in vitro and which along with other as yet unidentified factors may play a part in haemopoietic inhibition¹⁸⁻²⁰. There is usually an increase in plasma alanine transaminase and gamma glutamyl transferase, possibly caused by hairy cell infiltration of hepatic sinuses and portal tracts. A positive reaction for isoenzyme \5 of acid phosphatase which is resistant to treatment with tartrate (TRAP positive) is a characteristic feature of hairy cells^{3,17}. Only a small minority of cells in other lymphoproliferative disorders may give a positive TRAP reaction¹⁷. The neutrophil alkaline phosphatase score is elevated in patients who have a normal neutrophil count. Hairy cells show features of activated B lymphocytes^{21,22}. They also express aberrant immunologic markers of monocytes as well as activated T lymphocytes. Immunophenotyping shows strong surface membrane IgA and IgG expression and positive reactions with monoclonal antibodies to CD 19, CD20 and CD22, which are B cell markers. Positive reactions with FMC7, HC2, CD25 and CD11c antibodies are also usual^{4,22}. It is likely that at least 25% of cases of hairy cell leukaemia show clonal chromosomal abnormalities; chromosomes 14 and 12 are involved in the majority of these, but at present the breakpoints and association with oncogenes are not known²⁴⁻²⁷.

Diagnostic Criteria

Characteristic hairy cells seen on blood film examination associated with splenomegaly are virtually diagnostic²⁸. Trephine marrow biopsy showing typical histological changes and the demonstration of TRAP positive cells in blood or marrow together with appropriate immunologic cell surface markers confirm the diagnosis. Difficulty may arise when few or no hairy cells are found in the peripheral blood. The clinical conditions which should be considered in the differential diagnosis are CLL with isolated splenomegaly²⁸ splenic lymphoma with villous lymphocytes²³ prolymphocytic leukaemia, myelofibrosis and aplastic anaemia^{9,28}. If few or no hairy cells are seen on peripheral blood examination, marrow aspirate yields only a dry tap and the trephine biopsy is hypocellular, aplastic anaemia may be mistakenly diagnosed. The clinical findings and the results of the examination of the patient's blood film and bone marrow supported by the demonstration of appropriate cytochemical and immunologic markers establish the diagnosis in almost every case.

Treatment

The treatment of hairy cell leukaemia has changed dramatically in the last ten years. With the advent of effective chemotherapy, the aim of treatment has shifted from palliation to eradication of the disease. Until recently, patients with minimal or no symptoms were often left untreated. Symptomatic patients were frequently splenectomized at some stage of the disease and received supportive treatment including blood transfusion and antibiotics as necessary. Systemic treatment for hairy cell leukaemia should if possible be started well before the development of profound cytopenia or other major complications.

Splenectomy:

Until recently splenectomy was considered the treatment of choice for hairy cell leukaemia^{29,30}. In up to 50% of patients, blood counts return to normal after the operation, while amongst 30-40% of the remainder there is some improvement in at least one blood cell line. Although an occasional patient enters lasting remission, the majority relapse and the disease gradually progresses if no other treatment is given. Presumably splenectomy removes the main site of cell sequestration as well as removing a major part of the tumour bulk^{31,32}. It is sometimes followed by complete reversal of marrow findings, an effect which is difficult to explain. The response to splenectomy may be rapid, the platelet count recovering within two weeks of operation followed by increases in neutrophil numbers and haemoglobin over subsequent months. Non-responders have a poor prognosis without systemic therapy^{31,32}. Since the advent of systemic drugs the role of splenectomy has decreased, but the operation may still be considered in those who fail to respond to interferon, deoxycorymycin or

chlorodeoxyadenosine. It may also be considered in patients with symptoms due to massive splenomegaly.

Cytotoxic Chemotherapy:

Systemic chemotherapy with or without splenectomy was frequently employed before the introduction of interferon. Despite the occasional claims of good responses obtained with cytotoxic agents including chlorambucil, cyclophosphamide, anthracyclines, steroids and vinicristine alone or in combination, any beneficial effects were as a rule minimal and transient³⁴⁻³⁷.

InterferonAlpha:

In 1984, Quesada et al. reported seven patients treated with interferon alpha (IPN a) 3 million units/day by intramuscular injection for at least six months. Three patients achieved complete remission and four showed partial responses. These effects were maintained for many months after stopping the drug³⁹. Several investigators have confirmed Quesada's observations⁴⁰⁻⁴⁸. Interferon is effective in up to 80% of patients treated, 70% achieving partial remission and 5-10% a minimal response with improvement of only one blood cell type. Only a minority of patients (5%) enter complete remission⁴⁶. The type of alpha interferon used, whether the product of a cultured lymphoblastoid cell line or a recombinant DNA product appears to make little difference in the response to treatment. In patients who enter complete remission, the platelet count returns to normal within approximately two months of starting treatment. Haemoglobin may take four months and neutrophils four to six months to recover³⁹⁻⁴¹. Hairy cells decrease in the peripheral blood within four weeks of therapy. These improvements in peripheral blood count are the direct result of a gradual reduction in bone marrow infiltration accompanied by an expansion in haemopoietic tissue. Complete eradication of hairy cells from the marrow is rare. Marrow fibrosis usually persists despite long term treatment with interferon. The optimal dose and duration of treatment is not well defined⁴⁰⁻⁴⁸. The dose most commonly used for recombinant interferon alpha 2b (IFN alpha 2b) is 3 million units 3 times a week by subcutaneous injection for 12-18 months while for interferon alpha 2a (IFN alpha 2a) it is 3 million units daily by subcutaneous injections for the same duration. Low dose IFN 0.2 million units per square meter of body surface area (BSA) per day is less toxic but also less effective than "standard dose" IFN alpha⁴⁹. Higher doses are less well tolerated. A recent trial evaluated the duration of IFN treatment in two prospective groups⁴⁸. One group received the drug for 12 months and the other for 18 months. This study did not find significant differences in peripheral blood count in the two groups at the end of IFN treatment. The number of patients requiring retreatment in both groups was similar. The mechanism of action of IFN alpha is not fully clear. Its direct effects on hairy cells include modification of gene expression, induction of specific protein synthesis and modulation of cytokine production. These actions induce hairy cells to differentiate and make the cells resistant to growth factor stimulation. Interferon alpha also activates the production of natural killer (NK) cells⁵⁰. Interferon alpha is usually well tolerated at the "standard dose"⁴⁸. During the first week or so, many patients feel influenza like symptoms which may include fever, chills, headache, malaise, myalgia and tachycardia. Paracetamol 1 gram half an hour prior to each injection ameliorates these symptoms. It is often recommended that interferon should be injected at night so that the side effects are not as troublesome. On continued treatment these iatrogenic symptoms usually resolve. Myelosuppression is a frequent side effect and a proportion of patients receiving prolonged treatment complain of chronic fatigue⁴⁸. Other rarer side effects include dry skin, diarrhoea, constipation, depression, paraesthesia and loss of libido. Up to 30% of patients treated with IFN alpha 2a have been found to develop neutralizing antibodies⁵¹. These antibodies disappear on long term follow up despite continued IFN alpha 2a injections⁵¹. Neutralising antibodies are rarely formed against IFN alpha 2b^{52,23}. In cases where antibodies cause refractoriness to IFN alpha 2a, IFN alpha 2b can be used without cross reactivity; alternatively, treatment with deoxycoformycin or deoxyadenosine may be considered.

Deoxycoformycin (Pentostatin):

Deoxycoformycin (2-DCP) is an inhibitor of adenosine deaminase (ADA), an enzyme which is necessary for lymphocyte proliferation and functions. The exact mode of action of this drug against hairy cell leukaemia is not certain but there is evidence that lympho-cytotoxicity is mediated by the accumulation of deoxyadenosine triphosphate leading to ribonucleotide reductase inhibition⁵⁴⁻⁵⁶. . Breakage of single stranded DNA, depletion of NAD and inhibition of intracellular methylation may be produced by 2-DCP⁵⁶. Because of the high adenosine deaminase levels in lymphoid tissue, 2-DCP is active against hairy cell leukaemia and other lymphoproliferative disorders. Spiers et al. reported 2-DCP activity in hairy cell leukaemia in 2 patients and since then other groups of investigators have confirmed a high degree of activity against this disease⁵⁴⁻⁵⁶. The overall complete response rate is 50-80%²⁹. The response is quicker than to IPN and peripheral blood counts start to improve within two weeks of starting treatment. 2-DCP is active in both untreated and previously treated patients^{57,58}. The recommended dose is 4 mg per square meter of body surface area every 2 weeks for 8-10 cycles or until remission is achieved, followed by 2 more cycles as consolidation. Grever et al.⁵⁹ reported the results of a randomized trial comparing 2-DCP (4 mg per square meter of BSA every 2 weeks) with IFN alpha (3 million units 3 times a week subcutaneously) in 356 untreated patients. Of the patients in the 1PM arm, 11% achieved complete remission and 38% partial remission. In the 2-DCP arm 69% entered complete remission (CR) and a further 6% partial remission (PR). The relapse free survival was significantly longer in the 2-DCP arm; 50% of those who achieved CR relapsed at 10-19 months in the 1PM group while only 4% in the 2-DCP group relapsed after 13-37 months follow up. Side effects⁵⁴⁻⁵⁸ of 2-DCP include nausea, vomiting, skin rashes, keratoconjunctivitis, fever, renal impairment and neutropenia. It should be used with caution in patients with renal insufficiency and acute infections. Urba et al⁶⁰ have shown a fall in T lymphocytes in 13 patients treated with 2-DCP alternating with IPN. Both CD4 and CD8 count decreased to less than 200 cells/microlitre in all patients throughout 14 months of treatment and 6 months thereafter. Levels of CD4 and CD8 returned to normal in only 4 cases. Localized infections with herpes zoster virus have been reported during treatment.

2-Chlorodeoxyadenosine (Clidrabine):

2-Chlorodeoxyadenosine (2-CdA) is a purine analogue in which chlorine is substituted for a hydrogen atom at position 2 of the purine ring. This substitution renders the drug resistant to adenosine deaminase^{61,62}. As a result 2-CdA accumulates in the lymphoid tissues of the treated patients as chlorodeoxyadenosine triphosphate. The enzyme deoxycytidine kinase phosphorylates 2-CdA to chlorodeoxyadenosine triphosphate leading to DNA strand breaks in lymphocytes, decreased RNA synthesis and depleted energy production^{61,62,65}. It has been suggested that 2-CdA induces apoptosis (programmed cell death) in sensitive cells⁶¹. Its exact mechanism of action is not fully established, but like 2-DCP, it is active against many lymphoproliferative disorders. Precinical studies in lymphoblastoid cell lines have shown that both B and T cells are sensitive to 2-CdA, but T cell lines are affected by a lower concentration of the drug⁶⁶. Piro et al.⁶³ reported 86 patients who received single course of 2-CdA at a dose of 0.1 mg/kg/day by continuous intravenous infusion for seven days. These patients included both treated and untreated groups. Complete remission (CR) was obtained in 80%, while most of the remaining 20% achieved good partial remission (PR). Previous treatment did not affect the response rate. Tailman et al.⁶⁷ reported 26 patients treated with 2-CdA at 0.1 mg/kg/day for seven days as IV infusion; 15 of the 26 patients were previously untreated while 11 had had treatment with 1PM alpha or 2-DCF or had been splenectomized. After a single course of 2-CdA, 16 of the 20 evaluable patients were in CR, while 4 obtained PR. Of the 4 patients who showed a partial response, 3 had residual disease in the marrow and received a second course of 2-CdA; 2 of these subsequently achieved CR. In summary, 18 out of 20 evaluable patients (90%) attained CR after 1 or 2 cycles of 2-

CdA. Juliusson and Lilliemark⁶⁸ and Estey et al.⁶⁹ have also reported a CR rate of 80% after a single course of 2-CdA. These studies have shown that 2 CdA has a much greater activity against hairy cell leukaemia than any other form of treatment, with sustained complete response rates after a single course approaching 80%. Such results have attracted many research groups to use it as first line drug offering the possibility of cure. The major side effect of 2-CdA is a culture negative febrile episode occurring in 40% of neutropenic patients which it has been suggested may be due to lysis of hairy cells^{63,65,70}. Apart from this drug related effect, infections with candida, cytomegalovirus and other fungal infections have been reported as a cause of fever. 2-CdA has been reported to reduce the number of both B and T lymphocytes in the peripheral blood. CD4 and CD8 counts drop and may remain low for as long as 12 months after treatment⁶⁴. The encouraging results of treatment with 2-CdA will require long term follow up, to determine how often CR equals permanent cure. The long term consequences of immunosuppression following its use will also need further study.

Conclusion

Large randomized studies are needed to evaluate the results of combined therapy with IFN, 2-DCF and 2-CdA compared to those obtained using a single agent. The last 10 years have seen dramatic advances in the treatment of hairy cell leukaemia which have changed therapeutic intentions from palliation to cure. It remains to be seen whether complete remissions after treatment with 2-DCF and 2-CdA amount to permanent cure in a substantial proportion of patients.

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