Peripheral blood lymphocyte subpopulations in patients with primary proliferative and secondary polycythaemia

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SUMMARY Peripheral blood lymphocyte subpopulations were measured in 18 patients with primary proliferative polycythaemia and 13 patients with secondary polycythaemia. A decrease in numbers of suppressor T lymphocytes and an increase in the helper:suppressor T lymphocyte ratio was found in those with primary polycythaemia compared with normal subjects and patients with secondary polycythaemia. If other causes of an increased helper:suppressor ratio are excluded this variable may be useful in confirming the myeloproliferative nature of patients with erythrocytosis.

In 1983 Eridani et al reported a study of peripheral blood lymphocyte subpopulations in patients with primary proliferative polycythaemia and idiopathic erythrocytosis. They found that in both groups there was a decrease in total numbers of T lymphocytes, with a particular decrease in the suppressor lymphocytes compared with the numbers seen in normal controls, so that the helper:suppressor T lymphocyte ratio was increased.

We performed a study of peripheral blood lymphocyte subpopulations in patients with primary proliferative and secondary polycythaemia to determine whether the helper:suppressor T lymphocyte ratio differed between the two groups.

Material and methods

Selection of patients

New and follow up patients with primary proliferative and secondary polycythaemia attending the outpatient departments at the Northern General and Royal Hallamshire Hospitals, Sheffield were studied.

Primary proliferative polycythaemia

Primary proliferative polycythaemia was diagnosed in patients fulfilling the following criteria: raised haematocrit and increased red cell volume, as measured by the $^{51}$Cr dilution technique giving values greater than 25% of the expected value adjusted for height and weight; together with the presence of at least two of the following variables, splenomegaly, increased neutrophil and platelet counts, and an increased leucocyte alkaline phosphatase value. These criteria differ slightly from those laid down by the Polycythaemia Vera Study Group.

Our group contained 18 patients, 10 men and eight women, with a mean age of 63 years (range 31–89). Twelve of them had been diagnosed within the previous five years, and the remaining six had been diagnosed at varying times prior to this. Seven had well documented splenomegaly at some time during their disease; eight had received at least one injection of $^{32}$P; and another three had received intermittent courses of busulphan to control their disease over the time before the study started. A further three patients had been controlled by venesection only. The remaining three were included in the study at the time of diagnosis.

Secondary polycythaemia

Secondary polycythaemia was diagnosed in patients with the following criteria: haemoglobin equal to or greater than 19 g/dl; packed cell volume equal to or greater than 60% in men and haemoglobin concentration equal to or greater than 18 g/dl; packed cell volume equal to or greater than 55% in women and in patients having a clear cut disorder associated with secondary polycythaemia. It was impossible to verify a true polycythaemia by performing red cell volume studies in all these cases, but a haemoglobin concentration and packed cell volume above these values is unlikely not to represent a true erythrocytosis.

This group included eight patients with long stand-
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...ing chronic obstructive airways disease (seven men and one woman of mean age 62 years (range 52–70)); three patients with Eisenmenger's syndrome after congenital heart disease (two men, aged 29 and 33, and one woman aged 50); one man, aged 63, with polycystic kidneys; and one woman, aged 44, with obstructive sleep apnoea.

All patients with chest and heart disease resulting in polycythaemia induced by hypoxia had arterial oxygen pressure of less than 8·6 kilopascals and arterial oxygen saturations of less than 92%. Below these arterial oxygen values it is thought that hypoxia induced erythrocytosis is likely to develop.3

In all patients with secondary polycythaemia the erythrocytosis had been treated only by intermittent venesection.

SELECTION OF NORMAL SUBJECTS
A normal range of Tlymphocyte subset values and helper:suppressor Tlymphocyte ratios was established using 17 healthy medical laboratory staff, none of whom had features to suggest a diagnosis of primary proliferative or secondary polycythaemia.

LYMPHOCYTE SUBPOPULATIONS USING MONOCLONAL ANTIBODIES
Indirect immunofluorescence was carried out on lymphocytes isolated from peripheral blood samples by conventional methods,4 using four monoclonal antibodies: CD3 (T, p 19–29), Leu 4, and CD19 (B, p 95) Leu 12 (Becton Dickinson, Middlesex), CD4 (T, p 55), OKT-4 and CD8 (T, p 32–33) OKT-8 (Ortho-Diagnostics Systems). CD3 reacts with most peripheral blood lymphocytes that form rosettes with sheep red blood cells but does not react with B lymphocytes. CD19 reacts with all peripheral blood lymphocytes of B cell lineage as defined by the presence of κ and λ light chains but does not react with T lymphocytes. CD4 and CD8 react with the helper and suppressor subgroups of T lymphocytes, respectively.

Statistical analysis was performed on the data using Student's t test to compare the mean values of particular variables within each study group.

Patients included in this study were screened to exclude clinical conditions that are known to bring about an increased helper:suppressor T lymphocyte ratio.4–7 This screen comprised a clinical assessment and estimation of liver function tests, thyroid function tests, autoantibody screen, rheumatoid factor, direct Coombs' test, random blood glucose, and gastric intrinsic factor antibody.

Results
In each patient absolute values were obtained for total lymphocytes, total T lymphocytes, and helper and suppressor T lymphocytes from which the helper:suppressor T lymphocyte ratio was determined.

The mean (SD) absolute lymphocyte count for the normal subjects was 2080 (640) × 10⁶/1. For patients with primary polycythaemia it was 1710 (560) × 10⁶/1. There was no significant difference between any of these values. It can therefore be assumed that the observed changes in lymphocyte subgroup numbers in our groups of patients were not influenced by variation in absolute lymphocyte counts.

The table shows the mean (SD) absolute values for T3, T4, and T8 lymphocytes and the helper:suppressor T lymphocyte ratios within each study group.

The figure represents the helper:suppressor T lymphocyte ratios obtained within each study group.

There was no significant difference between the mean total T lymphocyte counts of normal subjects compared with those of patients with either primary or secondary polycythaemia. There was no significant difference between the mean numbers of helper T lymphocytes of normal subjects compared with those of either polycythaemic group.

Mean values for suppressor T lymphocytes were significantly decreased in patients with primary polycythaemia compared with those in normal subjects (p < 0·001), whereas there was no significant difference in this value between patients with secondary polycythaemia and normal subjects.

As a result of this decrease in suppressor T lymphocytes in the primary group the helper:suppressor T lymphocyte ratio was significantly increased in this group compared with that of normal subjects (p < 0·001) and patients with secondary polycythaemia (p < 0·01). The only

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<th>Table Mean (SD) absolute values for T3, T4, T8 lymphocytes and helper:suppressor T lymphocyte ratios</th>
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<td><strong>Lymphocyte populations (× 10⁶/1)</strong></td>
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Discussion

Our study confirms the finding of Eridani et al.\(^1\): helper:suppressor T lymphocyte ratios are significantly increased in patients with primary proliferative polycythaemia. In addition, we have shown that patients with secondary polycythaemia do not have raised helper:suppressor T lymphocyte ratios compared with those in normal subjects and that there is a significant difference in the ratio between primary and secondary polycythaemias.

The finding of reduced suppressor T lymphocytes and hence increased helper:suppressor ratios in our patients with primary polycythaemia seemed to be a persistent feature of the condition, remaining uncorrected by previous treatment, such as venesection, \(^{32}\)P injection, or busulphan chemotherapy.

A wide variety of conditions have been reported to cause an increase in the helper:suppressor T lymphocyte ratio. These include pernicious anaemia (with gastric intrinsic factor antibodies),\(^4\) newly diagnosed diabetes mellitus,\(^5\) Hashimoto's thyroiditis, rheumatoid arthritis, Sjogren's syndrome, Wegener's granulomatosis,\(^6\) autoimmune haemolytic anaemia, seronegative autoimmune chronic active hepatitis, myasthenia gravis, Berger's IgA deposit nephropathy, membranous glomerulonephritis, and acute episodes of multiple sclerosis.\(^7\) None of our patients with increased helper:suppressor T cell ratios had evidence of any of these disorders.

Patients often present to haematology clinics with increased haemoglobin concentrations, venous haematocrits, and red cell volume without any other features to suggest a definite diagnosis of primary or secondary polycythaemia.

Modan and Modan\(^8\) introduced the use of the term benign erythrocytosis for this type of patient in 1968. In 1979 Pearson and Wetherley-Main\(^9\) substituted the term idiopathic erythrocytosis. In their study of similar patients 40% progressed to primary proliferative polycythaemia within six years of follow up from time of diagnosis, so that in their experience the condition was not benign. Eridani\(^1\) showed that patients fulfilling the criteria of idiopathic erythrocytosis also had increased helper:suppressor T lymphocyte ratios similar to his patients with primary polycythaemia, thereby corroborating the theory that this condition is a primary proliferative disorder.

We conclude that the finding of an increased helper:suppressor T cell ratio of greater than 2.5 implies the likelihood of a primary proliferative disorder in patients with a true polycythaemia, provided other causes of an increased ratio can be excluded. Fourteen of our 18 patients with primary proliferative polycythaemia had helper:suppressor T cell ratios greater than 2.5 compared with none in the group with secondary polycythaemia, but we should point out that one of our normal control subjects did have a ratio of greater than 2.5.

The two main T cell subsets modulate erythroid progenitor cell growth in vitro. The helper T cells, with the cooperation of monocytes, stimulate progenitor cell growth, whereas the suppressor cells inhibit growth of these cells.\(^10\)\textsuperscript{-13}

Zoumbos et al.\(^14\) reported that 10 of 12 patients with aplastic anaemia had increased numbers of activated suppressor T cells compared with the numbers in control subjects. Furthermore, these cells were found to be releasing \(\gamma\)-interferon, which suppressed progenitor cell growth in vitro. The finding of reduced numbers of suppressor T cells in cases of primary proliferative polycythaemia may be important in the pathogenesis of this disorder. The relative excess of helper T lymphocytes may result in increased burst promoting activity on erythroid progenitor cells, an effect presumably mediated by a relative excess of growth factor substances released by activated helper cells. Helper T cell lymphokines may also bring about increased sensitivity of later erythroid precursor cells to erythropoietin, an in vitro finding that has been
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to erythropoietin, an in vitro finding that has been noted in patients with primary proliferative polycythaemia.\textsuperscript{15} 16

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References


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