HAEMATOLOGY

Chronic Granulocytic Leukaemia : Demonstration of the Philadelphia Chromosome in Cultures of Spleen Cells

AN abnormal marker chromosome in chronic granulocytic leukaemia was described by Nowell and Hungerford[1,2] and its occurrence widely confirmed[3–7]. This marker, the Philadelphia chromosome (Ph¹), occurs in almost all typical cases of chronic granulocytic leukaemia and appears to be an acquired abnormality specific for the disease. The Ph¹ is derived from a chromosome of pair 21 by loss of a variable proportion of the long arms.

It has been shown[8] that in untreated chronic granulocytic leukaemia all the dividing cells in direct preparations of bone marrow are Ph¹-positive. Apparently, megakaryocytes and erythroid precursors as well as granulocyte precursors carry the Ph¹. As haematological abnormalities are usually confined to the granulocytic series, it has been suggested[9] that the genetic material lost from the Ph¹ has immediate relevance to leucopoiesis only. It is postulated[8] that the erythroid, granulocytic, and megakaryocytic series have a common stem cell and the original deletion to produce the Ph¹ occurs in this stem-line. However, peripheral blood cultures in untreated cases yield mixed populations of Ph¹-positive and negative cells[4,11]. As the dividing cells in cultures of normal blood are almost certainly of lymphoid origin[10], it appears that the blood lymphocytes are Ph¹-negative and therefore must originate from a separate stem-line. Apart from cells of bone marrow origin and lymphocytes in the peripheral blood, cultured subcutaneous fibroblasts have been examined for the presence of the Ph¹ chromosome with negative results[3]. Only in the spleen are cells of both lymphocytic and bone marrow stem-lines to be expected to occur together, and so it is particularly important to study the cytogenetics of that organ in chronic granulocytic leukaemia. To our knowledge no such investigations have been reported. We have developed a method[12] for the cytogenetic study of lymphoid tissue; this technique was applied to spleen to obtain the results here reported.

The patient was a married woman, aged twenty-nine. Chronic granulocytic leukaemia, diagnosed 24 months previously, had been treated with busulphan, splenic irradiation, 6-mercaptopurine and cyclophosphamide. Resistance to all therapy had arisen and the disease had undergone acute transformation. Splenectomy was performed for massive splenomegaly and intractable thrombocytopenia. Splenic cell suspensions were cultured: (*a*) for 16 h without phytohaemagglutinin, (*b*) for 84 h with added phytohaemagglutinin. The chromosome count distribution was:

Chromosome No.	< 45	45	46	47	48	92–93	Totals
No. of cells (*a*)	7	8	26	5	1	0	47
(*b*)	7	12	53	16	0	2	90

On analysis, the dominant cell line was diploid and Ph¹-positive. A second line with 47 chromosomes due to the presence of a second Ph¹ was also identified. Excluding technically unsuitable cells, results of analysis as regards the presence of the Ph¹ chromosome were:

	Cells analysed	Ph¹+	Ph¹+ +	Ph¹−	Status uncertain
Preparation (*a*)	41	29	7	1	4
(*b*)	73	57	14	1	1

Thus Ph¹ negative cells accounted for only 2·4 per cent of the cells in 16-h culture and 1·4 per cent in 84-h culture. Splenic sections showed loss of normal architecture and absence of lymphocyte aggregations. A differential count of 500 cells in a splenic imprint showed 4 per cent of lymphocytes in a population of leukaemic blast cells and promyelocytes.

Thus almost all dividing cells from this patient's spleen were Ph¹ positive. This result, to be expected in the 16-h unstimulated culture, is surprising in the 84-h culture with phytohaemagglutinin, where Ph¹-negative cells of lymphoid origin might well be prominent. Possibly these cells failed to divide; however, splenic lymphocytes have been successfully cultured in this laboratory and elsewhere[13]. The initial small number of lymphocytes relative to leukaemic blast cells might explain the rarity of Ph¹-negative mitoses, but studies have shown that in cultures with phytohaemagglutinin normal cells may outstrip leukaemic cells[14–16]. A better hypothesis is that in advanced chronic granulocytic leukaemia all splenic stem cells, possibly including lymphocytic precursors, carry the Ph¹.

The presence of two Ph¹ chromosomes per cell has previously been observed in the chronic phase of chronic granulocytic leukaemia[17] and also in the phase of acute transformation[18–20], when it is more common. Further investigations of splenic cytogenetics in chronic granulocytic leukaemia during the chronic phase and after acute transformation are desirable.

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Stable Messenger RNA in Nucleated Erythrocytes

THE mammalian reticulocyte is capable of synthesizing haemoglobin for many hours after its nucleus has been extruded. Since the nucleus is the site of messenger-RNA synthesis[1], these cells must be using stable messenger for continued protein synthesis. In contrast to the red blood cells of mammals, erythrocytes of lower vertebrates and invertebrates are almost always nucleated. With the nucleus present these cells could conceivably synthesize protein on short-lived messenger templates. In order to determine whether nucleated red blood cells utilize short- or long-lived messenger-RNA, representative avian and reptilian erythrocytes were investigated by stopping DNA-dependent RNA synthesis with actinomycin *D* and following the course of protein synthesis in the functionally 'enucleated' cells.

Reticulocytosis was produced in young chickens and in adult turtles (*Pseudemys elegans*) by repeated bleedings; 15-day chick embryo erythrocytes (about 100 per cent reticulocytes) were used with identical results. Reticulocytes in turtle blood samples numbered about 10 per cent and in chick blood about 30 per cent. The red cells were separated by centrifugation, washed, and incubated in Waymouth medium with penicillin and streptomycin in