Proposals for the classification of chronic (mature) B and T lymphoid leukaemias

J M BENNETT,* D CATOVSKY,† M-T DANIEL,‡ G FLANDRIN,‡
D A G GALTON,¶ H R GRALNICK,§ C SULTAN,** THE FRENCH-AMERICAN-
BRITISH (FAB) COOPERATIVE GROUP

*From the *University of Rochester Cancer Center, Rochester, New York, United States of America; †Academic Department of Haematology, The Royal Marsden Hospital, London; ‡Institut de Recherches sur les Leucémies et les Maladies du Sang, Hôpital Saint-Louis, Paris, France; ¶MRC Leukaemia Unit, Royal Postgraduate Medical School, London; §Hematology Service, National Institutes of Health, Bethesda, Maryland, United States of America; **Service Central d'Hématologie-Immunologie, Hôpital Henri Mondor, Créteil, France*

SUMMARY Peripheral blood, bone marrow films, and bone marrow biopsy specimens from 110 patients, well characterised by clinical and laboratory studies, including electron microscopy, were reviewed, to determine proposals for the classification of chronic (mature) B and T cell leukaemias. On the basis of cytology and membrane phenotype the following disorders were defined: (i) B cell type: chronic lymphocytic leukaemia (CLL); CLL of mixed cell type, which includes cases with more than 10% and less than 55% prolymphocytes (CLL/PL), and a less well defined form with pleomorphic lymphocytes but less than 10% prolymphocytes; prolymphocytic leukaemia (PLL); hairy cell leukaemia (HCL); HCL variant; splenic lymphoma with circulating villous lymphocytes; leukaemic phase of non-Hodgkin's lymphoma (follicular lymphoma, intermediate, or mantle zone lymphoma and others); lymphoplasmacytic lymphoma with peripheral blood disease (mostly Waldenström's macroglobulinaemia); and plasma cell leukaemia. (ii) T cell type: T/CLL, which was differentiated from reactive T/lymphocytosis; T/PLL; adult T cell leukaemia/lymphoma; and Sézary's syndrome.

The recognition of distinct entities within the B and T cell leukaemias seems to have clinical and epidemiological connotations. It is hoped that these proposals may serve as the basis for further work, discussion, and improved management of patients.

For many years chronic lymphocytic leukaemia (CLL) was thought to be a highly variable disease and the heterogeneity of the morphological appearances of the lymphocytes seen in blood films was accepted as part of the variability. Although it came to be accepted that abnormal lymphoid cells could appear in the blood during the course of non-Hodgkin's lymphoma (NHL) and that these were sometimes distinctive enough to merit special names such as "lymphosarcoma cells" and "notched-nucleus cells" found in diffuse lymphocytic lymphoma and follicular lymphoma, respectively, and the Sézary cells of Sézary's syndrome, no systematic attempt was made to codify the whole spectrum in relation to the pathological and

clinical profiles. In the 1970s the advent of immunological techniques for characterising a whole range of membrane molecules, usually glycoproteins, and some cytoplasmic constituents, introduced a new dimension into our understanding of the immune system and the provenance and characterisation of many cell types. This new knowledge was rapidly applied to lymphoproliferative diseases.

The first broad characterisation into B and T cells soon led to the recognition that in most cases CLL affected B lymphocytes. Sézary cells were always found to be T cells, while the cells of hairy cell leukaemia (HCL), whose origin was controversial for many years, were finally accepted as B cells. Prolymphocytic leukaemia (PLL), first recognised on clinical and cytomorphological grounds,¹ was of B cell origin in most cases, but of T cell origin in about 25%.²

The discriminating power of immunological tech-

niques was greatly strengthened by the use of monoclonal antibodies against many cell surface epitopes. Some of these reagents were useful for identifying B and T cells while others helped to identify particular maturation stages of T lymphocytes. The application of morphological and immunological methods has reinforced the value of cytomorphology and has proved advantageous in increasing our understanding of the heterogeneity of B CLL itself. Nevertheless, in practice the cytological diagnosis of the chronic lymphoid leukaemias and allied conditions remains difficult because of their extreme variability. Certain cytomorphological features and immunological profiles are so strongly associated that a specific diagnosis can be suggested. In a few cases, however, a precise assignment cannot be made with confidence.

Cytogenetic studies are becoming increasingly important in the investigation of the lymphoid malignancies and point the way to the investigation of basic molecular defects in the genome. Some of the morphological types have been found to be constantly associated with particular chromosomal translocations—for example, the translocation t(14;18) regularly found in follicular lymphoma³ and which involves the fusion of an immunoglobulin coding gene with the bcl-2 sequence to yield a hybrid gene,⁴ trisomy 12 in CLL,⁵ t(11;14) in B cell PLL⁶ and inv(14) in T cell PLL.⁷

The conclusions resulting from the work so far accomplished by the FAB group are necessarily provisional but go some way towards establishing guidelines in the cytological characterisation of the chronic lymphoid leukaemias. The extent to which their more detailed categorisation will be clinically important remains to be worked out, although the broad correlations are already well established.

Markers for B and T lymphocytes

The identification of specific markers for B and T cells has provided the basis for two broad categories of lymphoid leukaemias. Two markers for B and T lymphocytes were the first to be categorised—membrane immunoglobulins (SmIg), also shown in the cytoplasm (CyIg), and a rosette test with sheep

erythrocytes, E-rosette, also recognised by monoclonal antibodies of the cluster of differentiation two (CD2). These methods and an increasing range of monoclonal antibodies are now essential for the classification of leukaemias and lymphomas. In addition to “pan-B” or “pan-T” reagents, which define the two main lines of differentiation, some antigens are restricted to some maturation stages and therefore fulfil a useful role for the subclassification. For example, B lymphocytes of CLL are characterised by a distinct pattern of reactivity⁸ that consists of the expression of SmIg (weakly) and of the CD5 antigen, high affinity for binding mouse erythrocytes (M-rosettes), and low expression of B maturation antigens such as those detected by monoclonal antibodies FMC7 or the CD22 group on the cell membrane.

The methods to be used and the choice of reagents will depend on the availability and experience of individual laboratories and are beyond the scope of this report. Viable cells are stained in suspension by immunofluorescence methods and observations made by flow cytometry or fluorescence microscopy. The detection of antigens or immunoglobulin on the cell surface and cytoplasm is also possible on fixed cell preparations—for example, those prepared by a cytocentrifuge, or on frozen sections of lymphoid organs prepared by immunocytochemical methods. The proposals for phenotyping B and T cell disorders are continuously improving with the introduction of new monoclonal antibodies.

B LYMPHOID LEUKAEMIAS

This group of disorders has well characterised clinical, morphological, and histological features. The defining criterion for diagnosis is the demonstration of a monoclonal population of B cells, shown by immunoglobulin light chain restriction, and evidence of B lineage differentiation shown by one or more specific monoclonal antibodies such as CD19, CD20, or CD24.⁹

The markers recommended for the diagnosis of chronic B cell leukaemias, listed in tables 1 and 2, are intended only as guidelines. The patterns of reactivity observed in the most common forms of B cell leukaemia and NHL with peripheral blood and bone

Table 1 Immunological methods for B cell markers

Reagent	Method*	Designation	Specificity
Anti-human Ig	Cell suspensions	SmIg	B lymphocytes (light chain restricted)
	Fixed cells	CyIg	
Mouse erythrocytes	Spontaneous rosetting	M-rosettes	B CLL cells
Monoclonal antibodies	Cells in suspension or fixed on slides	Immunofluorescence	(Table 2)
		Immunoperoxidase	
		Immunalkaline phosphatase	

*Cryopreserved cells may also be used.

Table 2 Monoclonal antibodies for the study of B cell disorders

CD No	Reactivity	Commonly used monoclonal antibodies
CD5	Mature T cells (strong) B CLL cells and some NHL cells (weak expression)	Leu1, T101, T1, OKCLL, UCHT2
CD10	Common ALL antigen, early B cells, and some NHL (follicular lymphoma)	J5, OKB-CALLA, VIL-A1, NU-N1, anti-CALLA
CD19	All B lymphocytes from early to late maturation stages	B4, Leu12
CD20	Most B lymphocytes	B1, Leu16, RFB7, NU-B2
CD21	Restricted to intermediate maturation stages	B2, RFB6, BA-5
CD22	Late B cells, hairy cells	B3, Leu14, TO15, RFB4, CLB/BLy1
FMC7*	Late B cells, hairy cells, B prolymphocytes	FMC7
CD24	Most B lymphocytes	BA1
CD25	Activated B and T cells; hairy cells	Anti-Tac, Tac1, IL-2R1
CD38	Activated B and T cells; plasma cells	OKT10
Anti-class II MHC antigens*	All B lymphocytes up to plasma cells; activated T cells and haemopoietic precursors	HLA-Dr, OKIa, GRB1, FMC4

*Not allocated to a particular cluster of differentiation; FMC7 is probably distinct from CD22, although findings in some of the B cell leukaemias suggest that both appear on the cell membrane at a relatively late stage of B cell maturation (table 3).

marrow disease are summarised in table 3. The phenotype of a chronic B cell leukaemia is immunologically mature and should be distinguished from that of early B lineage acute lymphoblastic leukaemias which are immunologically immature—for example, positive for the enzyme terminal deoxynucleotidyl transferase (TdT). In the other extreme plasma cells are associated with the loss of B cell antigens and class II molecules and the appearance of new antigens such as those detected by CD38 and other monoclonal antibodies such as PCA-1⁹ or BU11.¹⁰ Other reagents not included in table 3 are anti-HC2¹¹ and LeuM5 (CD11c)¹² which react with hairy cells. Because LeuM5 is also positive in monocytes, its value for detecting hairy cells may depend on the simultaneous demonstration of a B cell antigen, such as CD20 or CD24.

CLL is the B cell leukaemia which can best be defined by immunological phenotyping (table 3). Cells of some NHL (intermediate or diffuse small cleaved), however, share with CLL lymphocytes the expression of CD5 and have a weaker expression of SmIg than

other NHL and B cell PLL. "Deviation" from the typical CLL phenotype is seen in cases with an increased proportion of prolymphocytes or CLL/PL¹³ in which marker characteristics intermediate between CLL and PLL may be seen—for example, strong expression of SmIg and reactivity with FMC7.^{8,13,14} If four positive criteria for B cell CLL are set: weak SmIg, >30% M-rosettes, <50% CD5+ cells and <30% FMC7+ cells, a combination of three or four of these markers is seen in 80% of typical CLL, in 65% of CLL/PL cases, and in none of those with a diagnosis of B PLL.¹⁵ Two thirds of cases of B PLL have a phenotype quite different from that of CLL: strong SmIg, low M-rosettes and CD5, and >30% of FMC7+ cells.¹⁵

From the above it is apparent that immunological phenotyping provides useful information about cell lineage and state of B cell maturation. The final diagnosis, however, depends on the composite information derived from clinical features, cell morphology, histology and membrane markers. It should be recognised that even with optimal methods there

Table 3 Markers in chronic B cell leukaemias

Marker	CLL	PLL	HCL	NHL*		SLVL	Plasma cell tumours**
				Follicular lymphoma	Intermediate		
SmIg	Weak	Strong			Moderate	Strong	Negative
CyIg	-	-/+	-/+	-	-	-/+	++
M-rosettes	++	-	-/+	-/+	-/+	-	-
CD5	++	-/+	-	-	++	-	-
CD19/20/24	++	++	++	++	++	++	-
Anti-class II	++	++	++	++	++	++	-
FMC7/CD22	-/+	++	++	+	++	++	-
CD10	-	-/+	-	+	-/+	-	-/+
CD25	-	-	++	-	-	-/+	-
CD38	-	-	-/+	-/+	-	-/+	++

+ Indicate incidence at which a marker is positive in >30% of cells in a particular B cell tumour (+ + 80–100%; + 40–80%; —/+ 10–40%; — 0–9% of cases).

*Non-Hodgkin's lymphomas (NHL) with a high incidence of peripheral blood or bone marrow disease.

**Myelomatosis and plasma cell leukaemia; Waldenström's macroglobulinaemia cells show a similar phenotype except for sharing some features with PLL, HCL, and NHL (expression of FMC7, CD22, and some other B cell antigens).

are always some cases with atypical features that do not fit exactly in any of the named diagnoses, perhaps reflecting the great heterogeneity of the lymphoid system.

T LYMPHOID LEUKAEMIAS

There is considerable heterogeneity in this group of disorders because of the existence of many functional subsets and stages of maturation of T lymphocytes from which the leukaemia may originate. The reagents used for the diagnosis and classification of the T cell leukaemias are listed in table 4.

The main distinguishing feature between the immature (or thymic derived) and mature (or post-thymic) T cell disorders is the presence in the former, which comprises acute T lymphoblastic lymphoma, of TdT and thymic antigens, such as those recognised by CD1a. Neither TdT nor CD1a are found in chronic T cell leukaemias (table 5). Important for their consistency as pan-T markers are E-rosettes and CD2 and CD3. The pan-T reagents CD5 and CD7 are more consistently positive in thymic than in post thymic T cell malignancies (table 5).

Most types of mature T cell leukaemia involve CD4+ cells; cases of T CLL and persistent T lymphocytosis are often, but not always, CD8+ proliferations. Even within CD4+ disorders the neoplastic cells may represent different functional subsets. In addition, other reagents may add further diagnostic information—for example, CD25 is characteristically positive in adult T cell leukaemia/lymphoma (ATLL) cells and rarely in other T cell leukaemias. Monoclonal antibodies such as Leu7, CD16, and CD11b which react with larger granular lymphocytes and cytotoxic cells, including natural killer cells, can further identify rare types of T cell leukaemia, which often come under the designation of T CLL.

As with the B cell disorders, immunological methods are necessary to define T cell leukaemias and provide guidance to specific diagnoses, but are not

Table 5 Markers in chronic (mature) T cell leukaemias

Marker	T-CLL	T-PLL	ATLL	Sézary's syndrome
TdT	—	—	—	—
CD1a	—	—	—	—
E-rosettes	++	++	++	++
CD2	++	++	++	++
CD3	++	+	++	++
CD4	—	+	++	++
CD5	—	++	++	++
CD7	—	++	—	—
CD8	++	-/+	—	—
CD25	—	—	++	—
CD38	—	—	—	—

+ Indicate rate at which a marker is positive in >30% of cells in particular T cell leukaemias (++ 80–100%); -/+ 10–40%; — 0–9% of cases).

sufficient to define clinicopathological entities. For this, the immunological features need to be integrated with cytomorphological and histopathological findings. Furthermore, monoclonal antibodies cannot establish whether a T cell proliferation is clonal. This is now possible by analysis of the rearrangement of the T cell receptor β - and γ -chain genes¹⁶ and by chromosome studies.

Material and methods

Peripheral blood and bone marrow films from a total of 110 cases with a diagnosis of chronic B and T cell leukaemias were reviewed. We excluded from review cases of Burkitt's lymphoma/leukaemia and large cell transformation of T and B cell NHL. The group met first in May 1985 in London and analysed material from 33 cases previously circulated to examine the morphological spectrum of disorders. No information was then made available on clinical and cell marker studies. A preliminary classification proposal was established and evaluated with subsequent analysis of 59 new cases. These were discussed at a second workshop in Bethesda in April 1986. A set of slides from 20 cases in which there was no consensus on the diagnosis was reviewed again and discussed with the help of a television projection microscope. Formal diagnostic criteria, formulated after the immunological information and available in all cases, were also considered. In a substantial number of cases, bone marrow and lymph node biopsy specimens were also reviewed and in some cases of particular diagnostic difficulty, electron microscopy photomicrographs were available. In October 1987 material from another 18 cases was examined in London, with particular emphasis on the leukaemic phase of NHL.

CLINICAL-MORPHOLOGICAL DISEASE ENTITIES
The group agreed that it was necessary to examine the

Table 4 Monoclonal antibodies for the study of T cell disorders

CD No	Reactivity	Commonly used monoclonal antibodies
CD1a	Cortical thymocytes; T lymphoblasts	Leu6, T6, OKT6, NA134, NU-T2
CD2	Receptor for sheep erythrocytes; most T cells	T11, OKT11, Leu5
CD3	Mature T lymphocytes	OKT3, Leu4, UCHT1, T3
CD4	Helper/induced subset	OKT4, T4, Leu3, NU-TH/1
CD5	(Table 2)	
CD7	Mature and immature T cells	3A1, WT1, Leu9, OKT16
CD8	Cytotoxic/suppressor subset	T8, OKT8, Leu2, NU-T5/C, UCHT4
CD25	(Table 2)	

Classification of B and T cell leukaemias

B and T leukaemias separately. It was recognised that for some of them, such as CLL, HCL, or ATLL, the diagnosis could be made by morphology alone, but for others the B or T cell nature could not always be ascertained without the appropriate cell markers.

B CELL LEUKAEMIAS—MORPHOLOGY OF THE CELLS

The characteristics of the various types of lymphocytes seen in the most common chronic B cell leukaemias are summarised in table 6. Here the main distinguishing features between the small lymphocytes of typical CLL and the large lymphocytes and prolymphocytes seen in CLL of mixed cell type are emphasised. A subtle distinction can be made between the pleomorphic prolymphocytes seen in CLL/PL and the more uniform ones seen characteristically in B cell PLL. In the leukaemic forms of NHL cleaved or cleft cells are characteristic of follicular lymphoma, and cells of variable morphology and some degree of nuclear irregularity are a feature of other types of NHL.

Chronic lymphocytic leukaemia

Peripheral blood Defining criteria using the current staging systems for the disease suggest that a persistent lymphocytosis of $> 10 \times 10^9/l$ is sufficient for a diagnosis of CLL. In adults with lymphocytosis between 5 and $10 \times 10^9/l$ cell marker studies are necessary to confirm the presence of monoclonal B cells. In typical cases of CLL the cells are small with a conspicuous though narrow rim of cytoplasm (fig 1a). Both the nuclear and cytoplasmic outlines are regular, although a small degree of nuclear irregularity with a kidney shape or a small indentation is seen in some cases (fig 1b). The cytoplasm is homogeneous and weakly basophilic and granules are not seen. The nuclear:cytoplasmic ratio is high. The nuclear chromatin is characteristically dense with clumps of dark chromatin separated by narrow pale spaces. With the standard Romanovsky stains the nucleoli are either not visible or are inconspicuous and small. Some

morphological variation is seen from patient to patient. In some, the cytoplasm tends to be a little more abundant. When more than 10% of the lymphocytes are large or are prolymphocytes the diagnosis of mixed cell type CLL should be considered. Smudge cells are artefacts of blood films, which are not diagnostic, but nevertheless are characteristic of B cell CLL and in general tend to be more conspicuous with a high white cell count.

Bone marrow Aspirates are generally hypercellular and show lymphocytic infiltration. Bone marrow biopsy specimens are necessary to define the various patterns of infiltration and to exclude the diagnosis of NHL such as follicular lymphoma. The extent of infiltration, which may be interstitial, nodular, mixed or diffuse, correlates with the clinical state.^{17 18}

Lymph node biopsy Although lymph node disease in CLL is common, biopsies are not performed routinely. They are necessary in cases with low lymphocyte counts or when the membrane phenotype departs considerably from the typical pattern described in table 3, and, when the counts are high, if the morphology suggests a diagnosis of NHL.

CLL, mixed cell types

In several cases diagnosed as CLL the morphological features are different from those described above. Two types of this form of CLL may be found: (i) a mixture (dimorphic picture) of small lymphocytes and prolymphocytes ($> 10\%$ and less than 55%) designated CLL/PL; and (ii) a spectrum of small to large lymphocytes with occasional (less than 10%) prolymphocytes. There is currently no clear evidence for a biological disadvantage in the latter group with respect to typical CLL, but data are accumulating which suggest refractoriness to treatment and a worse prognosis for the CLL/PL group.^{19 20}

In two thirds of cases of CLL/PL the composite membrane phenotype of B CLL (see above) is found, and in one third only one or two of the CLL markers (usually CD5 or high M-rosettes) are demonstrable.¹⁵

Table 6 Types of leukaemic B lymphoid cells

Cell type (disease)	Size	Chromatin	Nucleolus	Cytoplasm	Other features
Small lymphocytes (CLL)	< 2 red blood cells	Clumped in coarse blocks	Absent	Scanty high nuclear:cytoplasmic ratio	Regular nuclear outline
Large lymphocytes (CLL, mixed cell)	> 2 red blood cells	Clumped	Inconspicuous or small	Low nuclear:cytoplasmic ratio, variable	Variable size
Prolymphocytes (PLL)	> 2 red blood cells	Clumped	One, prominent	Low nuclear:cytoplasmic ratio	Variable size
Pleomorphic prolymphocytes (CLL/PL)	> 2 red blood cells	Clumped	Central and prominent	Variable nuclear:cytoplasmic ratio	Variable size
Cleft cells (FL)	1-2 red blood cells	Homogeneously coarse	Absent or one or two inconspicuous	Scanty; not visible or narrow rim	One or two shallow or deep narrow nuclear clefts from angular base

Although clinical and laboratory features of CLL/PL are at least in some respects intermediate between those of CLL and PLL, marker studies suggest a closer affinity with CLL.^{13 15} The small lymphocytes of CLL/PL tend to have a larger volume than those of CLL²¹ and the prolymphocytes tend to be more pleomorphic than those seen in PLL (fig 1c). These cases are clinically heterogeneous and include patients with typical CLL which evolves into a more aggressive form, described as CLL in "prolymphocytoid" transformation,²² and others who present with an increased

proportion of prolymphocytes and whose course may not be progressive.

The cases in the second group of CLL of mixed cell type are less well defined and less common. These are characterised by larger cells than those seen in typical CLL, tend to have a lower nuclear:cytoplasmic ratio, and a trend towards increased cytoplasmic basophilia. A clear distinction between two cell populations by size (small and large) is not usually possible. The percentage of prolymphocytes is less than 10%. Histological studies of lymph nodes and bone marrow

biopsy specimens are important to distinguish these cases from some forms of NHL.

Prolymphocytic leukaemia

PLL is a distinct disorder characterised by a high white cell count and splenomegaly without lymphadenopathy.¹ The membrane phenotype of the prolymphocyte is quite distinct from that of CLL (table 3) but has similarities to that of other B cell leukaemias. The prolymphocyte is the predominant cell in peripheral

blood films (> 55%, usually > 70%) and is characterised (table 6) by its large size, round nucleus with a prominent vesicular nucleolus, relatively well condensed nuclear chromatin and a lower nuclear:cytoplasmic ratio than the small lymphocyte of CLL (figs 1d and e). These morphological features must be sought in well spread areas of blood films. The bone marrow infiltration is diffuse, not very different from that of CLL, or has a mixed interstitial-nodular pattern. Histological appearances of lymph nodes show diffuse

Fig 1 Peripheral blood films of B lymphoid leukaemias. (a and b) Chronic lymphocytic leukaemia (CLL). (c) CLL with more than 10% prolymphocytes. (d and e) Prolymphocytic leukaemia. (f and g) Hairy cell leukaemia (HCL). (h) HCL variant. (i and j) Splenic lymphoma with villous lymphocytes. (k and l) Follicular lymphoma. (m and n) Intermediate NHL. (o and p) Plasma cell leukaemia.

disease with or without a pseudo-nodular pattern.²³ The histological appearance of the spleen shows white and red pulp infiltration, with large proliferative nodules in the white pulp with a characteristic bizonal appearance—dense at the centre and lighter at the periphery.²⁴

Hairy cell leukaemia

The hairy cell is the outstanding morphological feature of this disease.²⁵ It has fine, irregular cytoplasmic projections or villi and is, overall, larger than most other lymphocytes. On peripheral blood films the cytoplasm stains light blue and has a poorly defined outline (figs 1f and g). Fine azurophilic granules are seen occasionally. Rod-shaped inclusions with a pale centre correspond to the ribosome-lamellar complexes seen at electron microscopic examination. The nuclear:cytoplasmic ratio is low and the nucleus is excentrically placed. The nucleus is oval, round, or

kidney-shaped. The nuclear chromatin has a fine dispersed pattern and nucleoli are inconspicuous, small, and usually single. The bone marrow is always affected in HCL but may be difficult to aspirate because of associated fibrosis. Material available from scanty aspirates or touch print preparations may be useful to identify hairy cells. Bone marrow biopsy sections are essential for diagnosis. These show variable degrees of infiltration by hairy cells which can be identified by the nuclear shape, the chromatin pattern, and the clear zone that separates one cell from another resulting from the abundant cytoplasm. The histological appearances of the spleen show a distinctive pattern of red pulp infiltration with the formation of pseudo-sinuses and widening of the pulp cords. Membrane markers (table 3) support the diagnosis of HCL, in particular the combined reactivity with CD25, LeuM5, and HC2. Tartrate-resistant acid phosphatase (TRAP) is a typical cytochemical finding.²⁶

Classification of B and T cell leukaemias

Hairy cell variant

This relatively rare disorder was described as having morphological features which are intermediate between hairy cells and prolymphocytes.^{27,28} The characteristic cells have an abundant cytoplasm, more basophilic than in hairy cells, but with similar villous projections, moderately condensed heterochromatin with a prominent nucleolus as in PLL cells, and a slightly higher nuclear:cytoplasmic ratio than typical hairy cells (fig 1h). The disease runs a chronic course with splenomegaly and a high white cell count (usually $> 50 \times 10^9/l$, a rare finding in typical HCL) without monocytopenia. As in HCL, the variant cells infiltrate the spleen mainly in the red pulp. The membrane phenotype is close to that of prolymphocytes. In contrast to typical hairy cells, HCL-V cells do not react with CD25 and HC2 but some cases are LeuM5 positive. Membrane IgG is often found on the cell membrane and in some cases SmIg cannot be shown.

Splenic lymphoma with villous lymphocytes (SLVL)

It has been recognised that some patients suspected on clinical and laboratory grounds (splenomegaly and circulating lymphocytes with villous projections) as having HCL, have, in fact, a predominantly splenic form of NHL, as established by analysis of the histological appearances of the spleen.^{29,30} A small monoclonal band is shown in the serum or urine in two thirds of cases. The white cell count is usually between 3 and $38 \times 10^9/l^{29}$ and the circulating lymphocytes, which comprise most of the cells, are larger than CLL lymphocytes and are close to the size of prolymphocytes. The nucleus is round or ovoid, has clumped chromatin, and in half of the cases has also a distinct, small nucleolus (fig 1i). The amount of cytoplasm is variable and is moderately basophilic. The main features are the presence of short villi, often localised to one pole of the cell. The nuclear:cytoplasmic ratio is higher than in hairy cells of HCL and HCL-V; a few cells show lymphoplasmacytic features (fig 1j). Membrane markers of SLVL cases are similar to those of B PLL. Unlike HCL cells, these villous lymphocytes do not react with HC2, CD25, LeuM5, or cytochemically with the TRAP reaction. The differential diagnoses to be considered are CLL, B PLL, HCL and HCL-variant. The histological appearances of the spleen show white pulp disease (in contrast to HCL and HCL-V), with or without clinically important red pulp infiltration.^{29,30} The bone marrow is not infiltrated in half of the cases; in others there is moderate to pronounced diffuse or nodular infiltration.

Leukaemic manifestations of NHL

Before the era of immunological markers several conditions (B and T derived) were broadly described as "lymphosarcoma cell leukaemia".³¹ These cases

were thought to represent de novo lymphoid leukaemias, but it is clear that these are cases of NHL which present in a leukaemic phase of their disease or which have progressed to a leukaemic phase. The most common form is follicular lymphoma in leukaemic phase.³²⁻³⁴ Spiro *et al* reported that 38% of cases with histologically confirmed follicular lymphoma presented with a lymphocytosis greater than $5 \times 10^9/l$ which was described as "CLL-like syndrome".³² In a significant proportion of such cases, however, the morphological features were different from those of CLL.

Whenever it is suspected that a lymphocytosis is associated with a NHL, bone marrow and lymph node biopsy sections are an essential part of the investigation. When referring to this group of cases, only those in which the disease presents in a leukaemic phase, which may be arbitrarily defined as $> 5 \times 10^9/l$ abnormal lymphocytes, will be considered here. This does not preclude the identification of a small percentage of NHL cells in the peripheral blood. Although the abnormal circulating cells may be derived from several histological subtypes of NHL,³³ the most common and best characterised form is the leukaemic phase of follicular lymphoma.³⁴ Cases of stage IV NHL with bone marrow disease³⁵ but no peripheral blood disease will not be discussed.

Leukaemic phase of follicular lymphoma

Although in the series of Spiro *et al* the highest lymphocyte count recorded was $30 \times 10^9/l$,³² cases with a higher white cell count ($45-220 \times 10^9/l$) have recently been recognised.³⁴ The morphological features of the circulating cells in follicular lymphoma (table 6) are small, cleaved lymphocytes (often smaller than CLL lymphocytes) with a high nuclear:cytoplasmic ratio and very scanty cytoplasm, usually confined to a thin rim visible between the concavities of the nucleus (figs ik and l). The nuclear chromatin is uniformly condensed without clumping. Prominent but usually very narrow nuclear clefts or fissures are seen in $> 30\%$ of cells; these clefts often arise in sharp angles from the nuclear surface and when they are deep they divide the nucleus in two. These features of the nucleus are even more apparent by ultrastructural analysis (fig 2). The cell outline is often angular and nucleoli are either absent or barely visible. Membrane markers (table 3) show differences with the findings in B CLL; SmIg is strong, the percentage of M-rosettes is low, FMC7 is positive, and CD5 is usually negative and CD10 is often positive. These findings are helpful when the cytological features described above are not typical and suggest a diagnosis of CLL. Bone marrow disease is common and typically shows a paratrabecular localisation of lymphocytic infiltration,³⁵ although diffuse infiltration can also be found.

Fig 2 Electron microscopy of two circulating cells from follicular lymphoma.

Intermediate NHL or mantle zone lymphoma

There are no detailed studies of the leukaemic manifestation of this lymphoma.³⁶⁻³⁸ The most common cell is of medium size, has condensed chromatin, an inconspicuous nucleolus with slight nuclear irregularities best characterised as indentations and clefts (fig 1m). The latter are not as pronounced or as deep as in follicular lymphoma cells. Larger cells with a high nuclear:cytoplasmic ratio and without a prominent nucleolus are seen (fig 1n). In a few cases the appearances are those given above for CLL of mixed cell types, except that most cases of intermediate NHL have a paucity of small lymphocytes. Bone marrow biopsy specimens show diffuse disease. The lymphoid population is monotonous and the cells are larger than CLL lymphocytes and tend to have an irregular nuclear outline. The above morphological criteria were derived from the examination of blood and bone marrow films from 12 cases in which there was a consensus for the diagnosis of NHL-intermediate type in leukaemic phase. The histopathological diagnosis of all these cases was made on the examination of lymph node biopsy specimens by independent observers. Membrane marker studies show CD5+ cells³⁶⁻³⁹ and SmIg staining of moderate intensity with μ and δ as the main heavy chain classes.³⁹ CD10 is positive when tested on all cell suspensions but negative by immunocytochemistry.

Lymphoplasmacytic lymphoma

These are cases in which monoclonal immunoglobulin bands are present in the serum and include Waldenström's macroglobulinaemia and also cases of SLVL, described above. In Waldenström's macroglobulinaemia the paraprotein concentrations are >20 g/l, while in SLVL the IgG or IgM paraprotein band concentrations are <20 g/l while the white cell count is usually $>10 \times 10^9/l$ in SLVL and $<10 \times 10^9/l$ in Waldenström's macroglobulinaemia.²⁹ The circulating lymphoid cells in Waldenström's macroglobulinaemia comprise a mixture of small and large lymphocytes, sometimes with an eccentric nucleus and pronounced cytoplasmic basophilia and morphological diversity. The bone marrow is often diseased in Waldenström's macroglobulinaemia with a mixture of cells of different nuclear maturity, some with dense chromatin and others with fine chromatin, and variable basophilia. A proportion of plasma cells and of mast cells is characteristic of Waldenström's macroglobulinaemia.

Plasma cell leukaemia (PCL)

PCL refers to patients with primary leukaemia and not the terminal phase of myelomatosis (multiple myeloma) in which abnormal plasma cells circulate in 2% of cases.⁴⁰ PLC is usually an acute illness sometimes resembling acute leukaemia. Hepato-

splenomegaly is more common and bone lesions are less common than in myelomatosis, while hypercalcaemia and renal failure are common. We recognise two morphological types of PCL. In one, the cells are small and range from lymphocytes with basophilic cytoplasm to plasma cells, and in the other they are blast-like cells. In the former but not in the latter type there is nuclear excentricity and a clear Golgi zone. Intense basophilia is a feature mainly of the small plasmacytic type (figs 1o and 1p). Membrane marker studies (table 3) are helpful in recognising the plasmablasts. Antigens normally found in B cells are lacking but new late B antigens appear. The presence of monoclonal C γ Ig and expression of CD38 are diagnostic. Ultrastructural analysis often discloses plasma cell features.⁴¹ Other laboratory investigations (Bence Jones proteinuria, hypercalcaemia) may also contribute towards a diagnosis of PCL.

T CELL LEUKAEMIAS—MORPHOLOGY OF THE CELLS

Once markers have assigned a lymphocytic proliferation to the T cell series (see above) the next step is to define the morphology of the cells, and it is on this that the classification will depend. The cells seen in the mature T cell leukaemias include lymphocytes with a range of morphological types: (i) large granular lymphocytes, characterised by abundant cytoplasm and azurophil granules; (ii) prolymphocytes with a prominent nucleolus, basophilic cytoplasm, and no azurophil granules; (iii) pleomorphic, polylobed cells; and (iv) small or large cerebriform cells. Lymphocytes with a mature morphology but without azurophil granules are present in normal peripheral blood but are very rarely seen as the predominant cell in T cell leukaemias. Such cells, however, may be seen in the small cell variant of T cell PLL but, on ultrastructural analysis, the latter always show a distinct nucleolus.⁴²

T cell lymphocytosis and T CLL

The upper limit of the absolute lymphocyte count in adults is about $4.8 \times 10^9/l$. Most normal lymphocytes are small to medium sized with a high nuclear:cytoplasmic and a densely clumped nuclear chromatin; nucleoli are usually indistinct. Most cells are agranular with a thin layer of basophilic or colourless cytoplasm. About 80% of the circulating lymphocytes are of T cell origin. Cases of reactive B cell lymphocytosis are extremely rare. More frequently, an absolute increase in the number of atypical T lymphocytes is seen as a result of several infective processes. Reactive lymphocytosis is self limiting and is rarely above $5 \times 10^9/l$. The most common causes are viral infections, Epstein Barr virus or cytomegalovirus in adults, and Bordetella pertussis in children in which very high counts may occur. The atypical cells have a lower nuclear:cytoplasmic ratio than normal, are often two to four

times the size of normal lymphocytes, and have variable cytoplasmic basophilia; sometimes the nucleus is irregular and resembles that of the monocyte but almost always has the coarsely clumped chromatin characteristic of lymphocytes.

In contrast to reactive lymphocytosis, chronic or persistent (> 3 months) T lymphocytosis of unknown cause occurs and further study shows the proliferation to be clonal. The lymphocytosis tends to increase gradually over the ensuing months or years and is often associated with cytopenias, usually neutropenia. If T lymphocytosis of $> 5 \times 10^9/l$ persists for over six months and consists of a relatively uniform population of lymphocytes, the diagnosis is likely to be T CLL; rarely, the white cell count is $> 20 \times 10^9/l$. In most cases the cells are large granular lymphocytes.⁴³⁻⁴⁵ These have a low nuclear:cytoplasmic ratio and show several fine or sometimes coarse azurophil granules in the abundant pale blue cytoplasm; occasional cells with the same morphology are agranular. The nucleus is round or oval, slight excentric, and has clumped nuclear chromatin; nucleoli are rare, as are smudge cells (figs 3a and b). These lymphocytes, which constitute 50–95% of the peripheral blood leucocytes, react strongly with cytochemical reactions for acid phosphatase and β glucuronidase, but weakly or not at all with α -naphthyl acetate esterase (ANAE).⁴⁶ Ultrastructural analysis shows that the azurophil granules are distinct structures designated as parallel tubular arrays.⁴⁷

In addition to cases with a chronic course and stable T lymphocytosis, are those with more bulky disease of liver and spleen and progressive course with rising white cell counts. In both types of T CLL the phenotype is that of the so-called T γ -lymphocyte with a spectrum of antigenic profiles; most cases with a chronic course have a CD3+, CD8+, CD4–, Leu7+, membrane phenotype (table 5). Cases with unusual phenotypes, representing granular lymphocytes rarely seen in normal peripheral blood—for example, CD4+, CD16+; CD8+, CD11b+, etc., tend to have a more aggressive evolution. DNA analysis with probes for the β and δ chain genes of the T cell receptor shows the genes to be in a rearranged configuration¹⁶ in most cases of chronic T cell lymphocytosis as defined above, thus justifying the term of T CLL or large granular lymphocyte leukaemia for this group of patients. Chronic T lymphocytosis can be distinguished from T CLL only by proving clonality by DNA¹⁶ or cytogenetic analysis in the latter condition.⁴⁵

Bone marrow aspirates in cases of T CLL show infiltration of variable degree; minor in the early stages, > 50% in later stages.^{44,45} Bone marrow trephine biopsy specimens show focal or more diffuse accumulations which do not displace the normal bone

marrow cells. In some cases a maturation arrest of the granulocytic series is seen and in others there is erythroid hypoplasia. The latter features are closely associated with the presence of neutropenia or regenerative anaemia.^{44,45}

T prolymphocytic leukaemia

About 20% of patients who present with clinical and morphological features of PLL have a T cell malignancy.² Within the mature T cell leukaemias T PLL may represent up to 40% of cases. Like B PLL, in T

PLL the white cell count is usually $> 100 \times 10^9/l$ at presentation but, in contrast, there are usually lymphadenopathy, skin lesions, and serous effusions as well as splenomegaly, and the course is aggressive. The circulating cells in half of the cases of T PLL resemble those of B PLL and unless cell markers are performed, it may be difficult to distinguish between these two disorders. In other cases T PLL cells have less cytoplasm and a higher nuclear:cytoplasmic ratio than B PLL cells⁴²; T prolymphocytes may also have an irregular nuclear outline (figs 3c and d). In some cases

Fig 3 Peripheral blood films of chronic T cell leukaemias. (a and b) T-CLL or proliferation of large granular lymphocytes. (c and d) T prolymphocytic leukaemia. (e, f, and g) adult T cell leukaemia/ lymphoma. (h) large Sézary cell. (i) Small Sézary cells or Lutzner cells.

the cells are small and the nucleolus is not easily seen by light microscopy. In general, T prolymphocytes have a deep basophilic cytoplasm which sometimes even suggests plasma cell differentiation. As a result of this morphological heterogeneity some cases have been described as T CLL with "helper" phenotype or T CLL of "knobby" cell type (when nuclear irregularities are present). The justification for a single disease entity in T PLL cases is threefold: (i) similar presenting features and aggressive clinical course; (ii) similar ultrastructural features, in particular a prominent nucleolus (fig 4) and the presence of large and dense localised granular structures⁴⁷ which correlate with the strong positivity with cytochemical reactions for most acid hydrolases, including ANAE⁶⁴; and (iii) a consistent chromosome abnormality, inv(14) in two thirds of the cases studied, with similar breakpoints in 14q11 and 14q32.⁷ Membrane markers (table 5) show that most T PLL are CD4+, CD8- proliferations; a minority coexpress CD4 and CD8 and a few are CD4-, CD8+.^{2,42} Unlike other mature T cell leukaemias, in particular those with a CD4+ phenotype, T PLL cells express strongly the CD7 antigen.

There is little information on the histopathological

features of T PLL. The few cases studied showed diffuse infiltration of the splenic red pulp with obliteration of the white pulp. In lymph nodes the infiltration is diffuse and affects predominantly paracortical areas. The bone marrow is usually diffusely infiltrated and shows increased fibrosis.⁴⁸

Adult T cell leukaemia/lymphoma

Over a decade ago the first reports of an unusual syndrome in Japanese adults appeared.^{49,50} The patients had manifestations of generalised lymphadenopathy associated with circulating abnormal lymphoid cells associated with hypercalcemia, bone lesions, skin lesions and bone marrow disease.

The white cell count ranges widely and the percentage of abnormal cells ranges from 10 to 80%.^{50,51} The cells vary considerably in size from that of a small lymphocyte to a large mononuclear cell (over 14 µm). The nuclear chromatin is relatively homogeneous and clumped or condensed. The nuclear outline has been described variously as convoluted or "clover leafed". This conveys the most striking feature of the cells, pronounced polymorphism (figs 3e, f, and g). Nucleoli are uncommon and when present are small. The cytoplasm is agranular and basophilic. Occasionally it

Fig 4 Ultrastructure of a prolymphocyte from a case of T-PLL.

is difficult to distinguish the cells from those seen in Sézary's syndrome; electron microscopy may help for this purpose (fig 5). There are also instances in which T PLL may be confused with ATLL because the prominent nucleolus normally seen in T PLL cells can be indistinct and the nuclear outline may be irregular.⁴² In addition to polylobed cells, some blastic cells with basophilic cytoplasm are almost always seen in blood films of ATLL. Positive cytochemical markers include acid phosphatase, β glucuronidase, and ANAE. The membrane phenotype is summarised in table 4. The cells are CD4+ and, unlike other T cell disorders, consistently express the receptor for Interleukin-2 (CD25).

There is a spectrum of clinical syndromes in ATLL.⁵² The most common, acute ATLL, presents with a high white cell count, hypercalcaemia, and the survival is less than one year. In the more chronic ATLL the white cell count is lower, there are fewer circulating abnormal cells, and the survival is more than one year. The bone marrow is often normal in

these cases. Finally, a group of patients have normal white cell counts and few non-specific symptoms, and a low proportion of abnormal T lymphocytes in the blood films. Evidence of clonality in such cases can be shown by molecular methods with appropriate HTLV-I probes. This condition has been designated as "smouldering" ATLL.⁵²

ATLL has a distinct epidemiology which relates to the distribution of its causative agent, the human type C retrovirus HTLV-I.⁵³ The association of HTLV-I with ATLL has been shown by serological and molecular studies. Almost all patients with ATLL have a high titre of antibodies to HTLV-I, and DNA analysis shows clonal integration of HTLV-I in the neoplastic ATLL cells. Most cases have been found in south western Japan,⁵⁰⁻⁵² the Caribbean region, and in Caribbean immigrants who reside in other countries.⁵⁴

Sézary's syndrome

This disorder is characterised by generalised exfoliative erythroderma and an epidermal infiltrate of

Fig 5 Electron photomicrograph of an atypical lymphocyte from a case of ATLL.

atypical mononuclear cells. The abnormal circulating cells in such cases are known as Sézary cells. Sézary's syndrome was the first disorder shown to be of T cell origin.^{55,56} In peripheral blood films there are two types of Sézary cell: large (fig 3h) and small (fig 3i). The large Sézary cells, with near tetraploid chromosome number, are less common. They are larger than neutrophils and sometimes also larger than monocytes. The nucleus is large and occupies four fifths of the cell with a round or oval profile and densely clumped chromatin. A grooved pattern is apparent and this corresponds to the cerebriform nuclear shape shown at ultrastructural level.⁴² Nucleoli are small and rarely visible. The cytoplasm shows a clear basophilia, has a narrow rim around the nucleus, and lacks azurophil granules.

The small cell variant of Sézary cell (also known as the Lutzner cell) is more common.⁵⁷ The cell is the size of a small lymphocyte (fig 3i) but has the same grooved nuclear chromatin pattern as the large Sézary cell (fig 3h). Lutzner cells, however, usually have a higher nuclear:cytoplasmic ratio than small lymphocytes and sometimes show cytoplasmic vacuoles (periodic acid Schiff positive) around the nucleus. Electron microscopical examination is useful for the morphological identification of small and large Sézary cells (fig 6). Bone marrow aspirates are either normal or show minimal infiltration; obvious disease is seen when the white cell count is high. Skin biopsy sections show the typical pattern of infiltration in the upper dermis with

epidermotropism and Pautrier microabscesses. The membrane phenotype of Sézary cells is that of a mature T lymphocyte with CD3+, CD4+, CD8— markers (table 5).

Discussion

In contrast to reliance on the cytomorphological and cytochemical features used in our previous proposals for the classification of acute leukaemia, other than megakaryoblastic leukaemia (M7),⁵⁸ we consider that a reproducible classification of the chronic B and T cell leukaemias required determination of the membrane phenotype of the neoplastic cells as well as a description of their morphological features. In both the B and T cell lineages several well defined disease entities can now be recognised in which the peripheral blood (leukaemic phase) and the bone marrow are affected. Some of the entities described here are well known but our proposals have emphasised the subtle phenotypic and morphological differences which allow disorders with specific clinical features, cell biology, and natural history to be defined more precisely than in the past. In addition to the morphological analysis of blood and bone marrow aspirates, the value of bone marrow trephine biopsies and, in the case of NHL in leukaemic phase, of the histological assessment of lymph nodes or spleen, has been emphasised.

Advances in treatment—for example, the specific response to α -interferon in HCL and not in the HCL—

Fig 6 Cerebriform appearance of a Sézary cell as seen by electron microscopy.

variant form (G Flandrin and D Catovsky, unpublished observation)—clearly justify the identification of the latter as a special subtype. Similarly, differences in the natural history and prognosis of CLL and PLL justify the separate identification of these forms of B cell lymphocytic leukaemia. Confusion with CLL and HCL often arises with some types of NHL in leukaemic phase. Again, the combined information of morphology and membrane phenotype helps in the identification of follicular lymphoma presenting with a leukaemic blood picture, and of splenic B cell lymphoma whose circulating cells are often confused with hairy cells.

The recognition of distinct entities within the T cell leukaemias has important epidemiological and pathophysiological connotations as illustrated by ATLL, the T cell leukaemia caused by HTLV-I and T-PLL where a specific chromosome abnormality, *inv*(14) (*q11q32*), is often found.⁷

In the acute leukaemias morphological and immunological features and cytogenetics have been

integrated to improve diagnostic precision and the objective classification of human haemopoietic malignancies.⁹ It is hoped that in the field of the chronic B and T cell leukaemias our proposals will serve as the basis for further work, discussion, and improved clinical practice.

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Requests for reprints to: Professor D Catovsky, Academic Department of Haematology and Cytogenetics, The Royal Marsden Hospital, Fulham Road, London SW3 6JJ, England.