DNA Content Analysis by Flow Cytometry and Cytogenetic Analysis in Mycosis Fungoides and Sézary Syndrome

DIAGNOSTIC AND PROGNOSTIC IMPLICATIONS

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ABSTRACT Flow cytometric (FCM) analysis of DNA content was performed on 82 lymph node and peripheral blood specimens from 46 patients with mycosis fungoides and the Sézary syndrome. Overall, 32 of the 46 patients (70%) had aneuploidy detected by FCM. Aneuploidy was present in 63% of the patients at the time of diagnosis before systemic therapy. In these patients, aneuploidy was frequently detected in blood and lymph node specimens scored as negative by cytology and histology, suggesting that unsuspected extracutaneous dissemination is present in many patients at the time of diagnosis.

Direct comparison with Giemsa-banded cytogenetic studies showed an excellent correlation of FCM results and cytogenetic chromosome number. However, FCM frequently detected a larger fraction of aneuploid cells, and mitogen-stimulation studies suggest this is the result of preferential stimulation of normal lymphocytes by phytohemagglutinin. Thus, mitogens with a preference for malignant T cells, such as staphylococcal protein A, should be used for cytogenetic analysis of malignant T-cell disorders.

At diagnosis, some histologically positive specimens contained only diploid cells by FCM and cytogenetic analysis. These patients had a more indolent clinical course than patients with aneuploidy. Aneuploidy was detected by FCM as either wide G_1 or as discrete aneuploid peaks. The presence of aneuploidy at any time in the clinical course implied a poor prognosis. Discrete hyperdiploid peaks were associated with large cell histology, early relapse, and aggressive clinical course. The development of hyperdiploidy at relapse was documented in four patients and was associated with a transition to large cell histology and a poor prognosis. Similar studies may elucidate differences in natural history and mechanism for transition in histology in other lymphomas and solid tumors.

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INTRODUCTION

Cytogenetic analysis, Feulgen microdensitometry, and, more recently, flow cytometry (FCM)[1] have been used for quantitative DNA analysis of normal and malignant human cells. Cytogenetic analysis is the most sensitive of these, and when used with modern banding techniques reveals abnormalities in as many as 80% of the patients with malignant lymphomas and leukemias and 90–100% of the patients with solid tumors (1–5). In some leukemias there is evidence that cytogenetic analysis provides prognostic information. In these instances, cytogenetic changes are associated with a transformation in the clinical course to a more aggressive nature with a poor prognosis (2–6). Cytogenetic analysis is, however, limited by requirements for live single cells with a relatively high proliferative rate; few cells are analyzed. Feulgen microdensitometry is not dependent on single-cell suspensions or proliferative state, and has been useful

[1] *Abbreviations used in this paper*: CTCL, cutaneous T-cell lymphoma; CV, coefficient of variation; DI, DNA index; FCM, flow cytometry; PHA, phytohemagglutinin.

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in demonstrating aneuploidy in a few patients with solid tumors and mycosis fungoides (7, 8). This type of analysis is limited to small numbers of cells.

FCM can rapidly analyze DNA content of large numbers of cells and is not dependent on the proliferative state of a tumor (9–11). Because living cells are not essential for the analysis, the cytoplasm can be lysed, allowing for easy cell dispersion (10). In addition, the proliferative state of the tumor (fraction of cells in the G_1, S, and G_2 + M phases of the cell cycle) may be analyzed (12). FCM has demonstrated aneuploidy in patients with leukemias, lymphomas, and solid tumors (13–17).

Cutaneous T-cell lymphomas, including mycosis fungoides and the Sézary syndrome, are malignant disorders of thymus-derived lymphocytes (T cells) (18–20). The peripheral blood and lymph nodes of cutaneous T-cell lymphoma (CTCL) patients frequently contain readily accessible T cells that can be subjected to FCM and cytogenetic analysis. We have performed FCM analysis of DNA content on samples from 46 patients with CTCL and compared these results with cytogenetic analysis on these same samples. FCM analysis demonstrated aneuploidy in 70% of the patients but in none of the 18 control samples. The FCM abnormalities showed excellent correlation with the cytogenetic results and provided pertinent diagnostic and prognostic information.

METHODS

Patients and controls. Analyses of DNA distribution patterns were performed on 18 controls and on 82 samples from 46 patients with CTCL. The control specimens consisted of peripheral blood samples from 16 normal volunteers and lymph node biopsies from 2 patients with benign chronic dermatoses. The median patient age was 53 yr with a range of 26–75 yr. There were 29 males and 17 females; 34 patients were Caucasian and 12 were Black. The 46 CTCL patients included 21 patients studied only at diagnosis, 11 at diagnosis and remission, 4 at diagnosis and relapse, 2 at diagnosis, remission, and relapse, 1 only in remission, and 7 only in relapse. Six of the eight patients studied only in remission or relapse had previously received systemic chemotherapy and one had previously received whole body electron beam irradiation. These seven patients and all others with repeat studies in remission or relapse had not been treated within 4 wk of FCM analysis.

Sample collection. Lymph node specimens, obtained by surgery, were immediately placed in Eagle's medium (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.). The lymph nodes were finely minced and passed through a 60-gauge stainless-steel mesh. The cells were resuspended in calcium and magnesium-free phosphate-buffered saline (Gibco Laboratories). Cell viability, as determined by trypan blue dye exclusion, was in excess of 90% in every instance. Peripheral blood specimens were collected in preservative-free heparin and subjected to Ficoll-Hypaque density separation. The mononuclear cells from the interface layer were collected and washed with phosphate-buffered saline.

FCM. For immediate DNA analysis, lymph node or peripheral blood cells were stained with 5 mg/100 ml propidium iodide (Sigma Chemical Co., St. Louis Mo.) in 0.1% sodium citrate by the method of Krishan (10). When DNA analysis was to be delayed, the cells were fixed in 50% ethanol and stored at 0–4°C. Immediately before analysis, the fixed cells were stained with propidium iodide after RNase treatment, according to the method of Crissman and Steinkamp (9). The DNA content of 50,000–100,000 cells was measured in a Coulter TPS-1 cell sorter (Coulter Electronics Inc., Hialeah, Fla.). Peripheral blood mononuclear cells obtained from a normal volunteer undergoing leukophoresis for granulocyte transfusion therapy were first analyzed as a diploid standard. The electronics of the instrument were adjusted so that the G_1 peak from the diploid standard was in channel 30 or 40 (128 total channels). With the same instrument settings, the patient specimen was analyzed subsequently. Finally, a mixture of specimen and diploid standard, which were stained together, was analyzed with the same instrument settings. Cell-cycle stage distribution analysis of the DNA histogram was conducted by a planimetric integration method recently described by Ritch et al. (21). To determine the degree of skewness of the G_1 peak, the coefficient of variation (CV) was determined for each sample from the formula $CV = (HM \times 100) \div (V \times 2.35)$, where HM is the width of the G_1 peak at half maximum and V is the modal channel number of the G_1 peak. To determine whether the G_1 peak of the sample was more skewed than the diploid standard, the CV ratio was calculated for each sample. The CV ratio was defined as the CV of the specimen to the CV of the diploid standard. To determine the ratio of DNA content of aneuploid cells to diploid cells, the DNA index (DI), defined as the ratio of the modal channel number of the aneuploid peak to the modal channel of the diploid peak, was calculated in each instance when discrete peaks were present.

Cytogenetic analysis. Peripheral blood buffy-coat cells or lymph node suspensions, were cultured for 1, 2, or 3 and 5–7 d with or without phytohemagglutinin (PHA) stimulation (22, 23). Chromosome-banding techniques were applied to one-half of the available metaphases and the resulting cells karyotyped according to the rules established by the Paris Conference (24, 25). When possible, at least 30 cells were analyzed from each specimen. The cytogenetic results were considered to be abnormal only when two or more cells contained structural abnormalities, when two or more cells had hyperdiploid chromosome numbers, or when three or more cells had hypodiploid chromosome numbers and were missing the same chromosome. Details of the cytogenetic results from these patients have been published elsewhere (26).

Histology and cytology. Using previously published criteria (27), Wright-Giemsa-stained peripheral blood smears were examined by Dr. G. P. Schechter for the presence of atypical convoluted lymphocytes ("Sézary cell"). Histologic interpretation of H. and E. stained lymph node sections was performed by one of the authors (A.G.). The lymph nodes were considered to be involved by lymphoma when the nodal architecture was partially or completely effaced by tumor cells or when numerous large clusters of Sézary cells were seen in the paracortical areas of the nodes.

Mitogen stimulation. Lymphocyte transformation in response to mitogen stimulation was measured by the cellular incorporation of ^{3}H-labeled thymidine. Peripheral blood mononuclear cells, isolated by density centrifugation, were plated in six replicate wells of Falcon flat-bottomed microtiter II tissue-culture trays (Falcon Labware, Div. of Becton Dickinson & Co., Oxnard, Calif.) at a concentration of 2×10^5 cells/100 μl culture medium (RPMI 1640 medium; Gibco Laboratories) supplemented with 20% fetal bovine

serum. Mitogens were added directly to the wells in 100 μl of culture medium. The mitogens tested and concentrations used were PHA (PHA-M 2%; Gibco Laboratories) and staphylococcal protein A 250 μg/ml (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). After incubation for 3 d at 37°C in a humidified atmosphere of 5% CO_2 in air, 1 μCi of ^{3}H-labeled thymidine (New England Nuclear, Boston, Mass.; 6–7 Ci/mmol) in 50 μl RPMI 1640 medium was added to each well 6 h before harvesting. Cells lysed with distilled water were collected on glass-fiber filter paper with a cell collector (Brandell, Rockville, Md.). The filters were placed in glass minivials containing 5.0 ml Aquasol liquid scintillation fluid (New England Nuclear) and their radioactivity determined with a Beckman LS-250 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Duplicate specimens exposed to each mitogen were examined for chromosome counts.

RESULTS

Three types of DNA abnormalities were present. (*a*) The first was distinct aneuploid peaks clearly distinguishable from the G_1 or G_2 + M peaks (Fig. 1). The position of the aneuploid peaks relative to diploid cells was always determined by the addition of diploid standard cells. This demonstrated that all samples with discrete aneuploid peaks also contained some cells with diploid amounts of DNA. Discrete aneuploid peaks were not present in any of the 18 control specimens. (*b*) The second was widened or skewed G_1 peaks that were an indication of near diploid numerical chromosome abnormalities (Fig. 2). The range and mean CV of the various specimens were: diploid standard lymphocytes, range 3.1–5.7, mean 4.6%; normal controls, range 3.1–5.8%, mean 4.5%; CTCL samples, range 3.9–12, mean 6.6%. Because the CV of the diploid standard varied slightly from day to day, the CV of the specimen was compared with the CV of the diploid standard performed the same day (CV ratio) to determine whether the width of the G_1 peak was increased. The CV of the normal control samples was never >12% more than the CV of the diploid standard (CV ratio ≤ 1.12). To minimize the number of discordant results based on a comparison of FCM and karyotypic results (see below and Table I), a specimen was arbitrarily considered to have an increased CV ratio when the CV from the patient specimen exceeded the CV of the diploid standard by >25% (CV ratio > 1.25). (*c*) The third was an increased fraction of cells in S phase. The control subjects had an average S fraction of 1.0% in the peripheral blood with a range of 0.1–3.0%. Control lymph nodes had S fractions of 0.1 and 4.1%.

Aneuploidy was detected by FCM analysis in 32 of the 46 CTCL patients (70%) and in none of the 18 controls. Aneuploidy was determined by the presence of discrete aneuploid peaks in 19 patients and wide G_1 peaks in 13 patients. Aneuploidy was found most

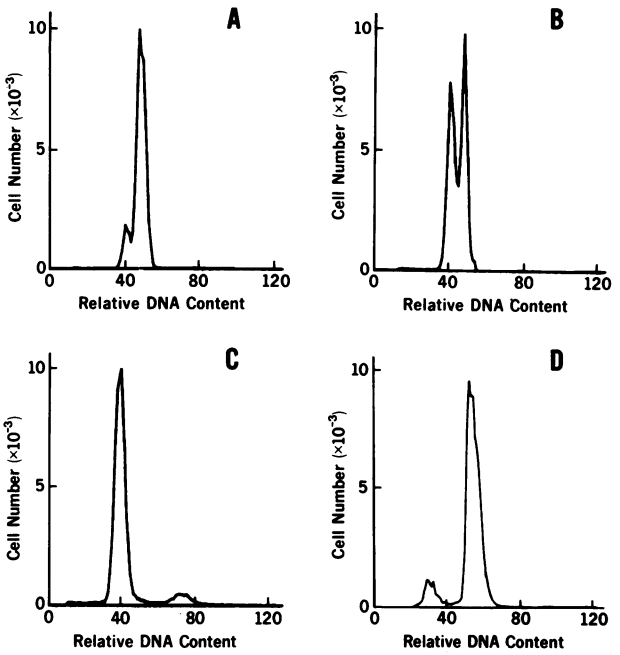

FIGURE 1 DNA histograms from the peripheral blood of three patients with discrete aneuploid peaks. In panels A, B, and C, the instrument electronics were adjusted so that the diploid standard was channel 40, whereas the diploid standard was channel 30 in panel D. Panel A shows the results from specimen 6 (Table IV), and panel B shows the result of a 2:1 mixture of this specimen and diploid standard lymphocytes. This demonstrated that the peak on the right was hyperdiploid with a DI = 1.20. Panel C shows the DNA histogram from specimen 2, with a discrete peak at channel 72 (DI = 1.80). Panel D shows the DNA histogram from specimen 4 with both a subtetraploid peak (DI = 1.70) and smaller hypodiploid peak (DI = 0.9).

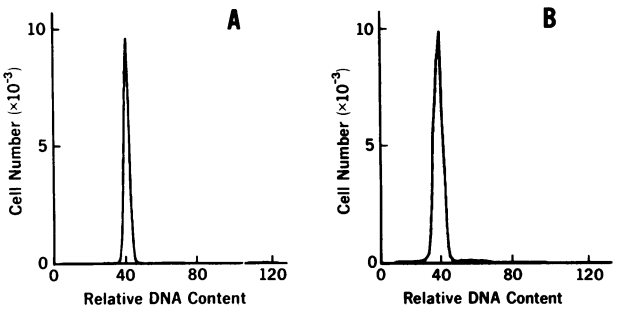

FIGURE 2 DNA histograms from the peripheral blood of a normal control (panel A) and from the peripheral blood of specimen 2 (Table I) (panel B). The electronics were adjusted so that the diploid standard lymphocytes (CV = 3.1) were in channel 40. The normal control lymphocytes had a CV of 3.1, whereas the CV from the sample was 5.1. Thus, the CV ratio was 1.0 for the normal control and 1.58 for the patient, indicating a widened G_1 peak. In this patient, cytogenetic analysis revealed that 44% of the metaphases were diploid and 56% of the metaphases were near diploid with 41–48 chromosomes.

TABLE I

Correlation of Cytogenetic Results and FCM Results in Samples without Discrete Aneuploid Peaks

FCM and CG abnormal*			FCM and CG normal (n = 5) or discordant (n = 3)		
Sample	FCM CV ratio	CG aneuploid (chromosome range)	Sample	FCM CV ratio	CG aneuploid (chromosome range)
		%			%
1	1.75	87 (42–47)	13	1.0	0 (46)
2	1.58	56 (41–48)	14	1.14	0 (46)
3	1.36	52 (43–47)	15	0.86	0 (46)
4	1.50	45 (42–48)	16	0.96	0 (46)
5	1.40	39 (43–47)	17	1.0	0 (46)
6	1.55	16 (44–49)	18	1.0	20 (42–45)
7	1.30	16 (42–47)	19	1.0	16 (45–48)
8	1.47	14 (42–48)	20	1.35	0‡ (46)
9	1.50	12 (44–48)			
10	1.47	10 (43–47)			
11	1.47	8 (44–47)			
12	1.28	16 (44–48)			

* CG, cytogenetic.

‡ This patient had all normal metaphases in the lymph node but, in the peripheral blood, 20% of the metaphases had near diploid numerical abnormalities.

frequently in patients with disease progression or relapse and least frequently in patients in remission (Table II). At the time of diagnosis, 38 patients were studied; 24 (63%) had aneuploidy, including 14 with a wide G_1 peak and 10 with discrete aneuploid peaks. The patients with aneuploidy were more likely to have extracutaneous disease, more advanced skin lesions (tumor or generalized erythroderma), more frequent relapse, and death as the result of disease (Table III and Fig. 3). Discrete aneuploid peaks were more commonly associated with these adverse clinical features than wide G_1 peaks.

14 patients were studied while in remission; aneuploidy was present in four patients (29%) (Table II). Previous studies had been performed in 13 of these patients; 6 initially aneuploid (wide G_1 peak) became normal; 3 remained normal; 4 remained aneuploid. At the time of disease relapse or progression, 12 of 13 patients (92%) had aneuploidy. Six of these patients previousy had been studied; four developed hyperdiploid aneuploid peaks not initially present (Fig. 4), one retained a hyperdiploid peak that increased its fraction of aneuploid cells, and one, initially diploid, developed aneuploidy with a wide G_1 peak. Seven other patients were studied only at relapse; five had discrete aneuploid peaks, one had a wide G_1 peak, and one was normal. 8 of the 13 patients studied at the time of disease relapse or progression have died; all 8 had aneuploidy, including 6 with discrete aneuploid peaks and 2 with wide G_1 peaks.

TABLE II

Comparison of FCM Results with Time of Study

Time of study	FCM results			
	Sample	Normal	Increased CV ratio (wide G_1 peak)	Discrete aneuploid peak
Diagnosis	38	14	14	10
Stable/remission	14	10	2	2
Relapse	13	1	2	10

The average percentage of cells in S phase in the peripheral blood of CTCL patients was 1.0% and was not different from normal controls (1.4%). Two CTCL patients had increased percentages of cells in S phase in the peripheral blood (4.1 and 12%); mitoses were observed in the peripheral blood of the latter of these patients. In the lymph nodes, CTCL patients had an average of 10% of cells in S phase and 12 patients had >10% of cells in S. Each patient with increased cells in S phase also had aneuploidy.

Correlation of cytogenetic and FCM results. Cytogenetic analysis was performed on the same sample analyzed by FCM in 30 instances and the results are compared in Tables I and IV. Aneuploidy was detected by cytogenetic analysis in 24 of the 30 specimens (80%) and by FCM in 23 of the 30 specimens (77%). Both the cytogenetic analysis and DNA histograms revealed aneuploidy in 22 samples, and both were normal in 5 samples. The FCM abnormalities in these 22 positive specimens were discrete aneuploid

TABLE III

Clinical Correlations with FCM Results in Patients Studied at Diagnosis

FCM result	Number of patients						
	Sample	Extra-cutaneous*	Plaques	Tumor	Generalized erythroderma	Relapse free	Dead from disease
Discrete aneuploid peak	10	8	2	4	4	4	3
Increased CV ratio (wide G_1 peak)	14	8	8	2	5	4	1
Normal	14	3	9	3	2	10	0

* Lymph node or visceral involvement.

peaks in 10 and an increased CV ratio in 12. Chromosome analysis revealed numerical abnormalities consistent with the DI of the discrete peak in each of these 10 samples (Table IV). Giemsa-banding analysis was performed on 8 of these 10 specimens, and clonal abnormalities were detected in 5. The other three specimens had numerous structural abnormalities, but no single abnormality was present in all cells. The 12 patients with wide G_1 peak all had near diploid chromosome numerical abnormalities (43–49, excluding 46) that were present in an average of 31% of the examined metaphases. Both hyperdiploid and hypodiploid cells were present in each instance, as were structural abnormalities. However, none of the samples had clonal abnormalities.

The FCM and cytogenetic results were discordant in only three samples (Table I). In one lymph node sample, FCM was abnormal with a CV ratio of 1.35, whereas the cytogenetics revealed only diploid metaphases. However, simultaneous cytogenetic analysis of the peripheral blood revealed that 20% of the metaphases contained near diploid numerical abnormalities. We conclude that the CTCL cells in the lymph nodes failed to replicate in this sample (see below). Chromosome analyses were abnormal with near diploid numerical abnormalities in two samples (16 and 17% of examined metaphases) when the FCM results were normal.

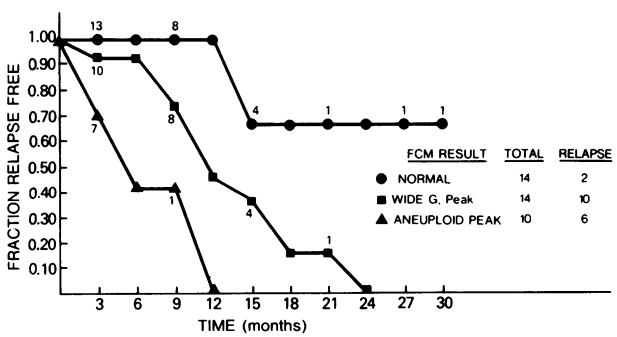

FIGURE 3 Actuarial relapse-free survival of 38 patients who were studied at diagnosis, including 14 patients with normal FCM results, 14 patients with a wide G_1 peak, and 10 patients with discrete aneuloid peaks. The numbers at times 3, 9, 15, and 21 mo represent the number of patients at risk at each time point.

Although there was a good correlation of FCM and cytogenetic results, the fraction of aneuploid cells determined by each method was strikingly different in 4 of 10 samples with discrete aneuploid peaks (Table IV; samples 4, 6, 8, and 10). In three of four samples, the fraction of aneuploid cells determined by FCM was larger than the fraction determined by karyotypic analysis. To investigate these differences, the cells from one of these patients (patient 6) were stimulated with PHA, the standard mitogen for cytogenetic analysis, and staphylococcal protein A. The FCM and cytogenetic abnormalities before and after mitogen stimulation are shown in Table V. In the absence of mitogen stimulation, FCM demonstrated that 86% of the cells were hyperdiploid. No metaphases were present for cytogenetic analysis. In the presence of PHA, the mitogenic response was poor (43, 578 cpm) and only 40% on the metaphases were hyperdiploid. In contrast, staphylococcal protein A was markedly mitogenic (147,026 cpm) and 80% of the metaphases were hyperdiploid.

Correlation of cytologic and histologic results with FCM. The results of FCM analysis are compared with the cytologic and histologic results in all 82 CTCL specimens in Table VI. The peripheral blood cytology was positive in 23/49 specimens (47%) and lymph node histology was positive in 22/33 specimens (67%). Aneuploidy was also found more frequently in lymph nodes (24/33) than in peripheral blood (25/49). Aneuploidy was detected by FCM in 36 of the 45 specimens (80%) with a positive histology or cytology and in 13 of the 37 histologically negative specimens (35%). Discrete aneuploid peaks were almost always associated with postive histology (21/24). In contrast, wide G_1 peaks were distributed nearly equally between histologically negative and positive specimens. Seven patients had a cytologic diagnosis of large cell variant of CTCL. All seven had discrete hyperdiploid peaks.

DISCUSSION

Aneuploidy was detected by FCM in 70% of the CTCL patients in this study, including 63% of the untreated

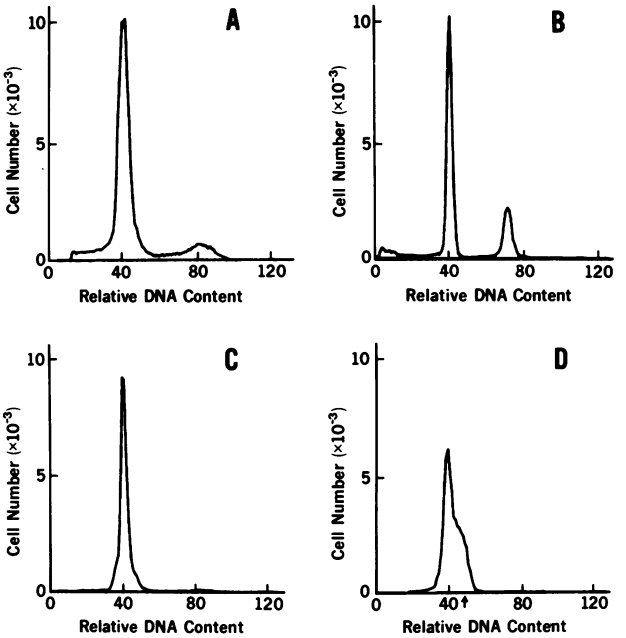

FIGURE 4 Sequential DNA histograms from two patients at diagnosis and at relapse. Panel A shows the histogram from the lymph node of patient 4 (Table I) at diagnosis. As in all proliferating tissues, including normal lymph nodes, two peaks are evident. The first peak (modal channel 40) is large and consists of G_1 cells, whereas the second, smaller peak (modal channel 80) is comprised of G_2 + M cells. Note the modal channel of the second peak (G_2 + M) is exactly twice that of the first peak (G_1). Cells with intermediate DNA contents between the two peaks are cells in the S phase. This histogram can be contrasted to those in Figs. 1 and 2 and taken from the peripheral blood, which is generally a nonproliferating compartment. Hence, the S and G_2 + M phases are not readily apparent. In panel A, discrete aneuploid peaks are not evident but the G_1 peak is abnormally wide with a CV ratio of 1.50. Karotypic analysis revealed that 45% of the metaphases had near diploid numerical chromosomal abnormalities and the node was histologically involved. At the time of disease relapse 24 mo later, a discrete hyperdiploid peak with a DI of 1.78 had developed (panel B). The modal channel number of this peak (channel 71) confirms the appearance of an aneuploid population of cells not present in the previous lymph node sample. Relatively few cells in S phase or G_2 + M phase are present. Panel C shows the DNA histogram from the lymph node of patient 12 (Table I) at diagnosis when the CV ratio was 1.28, indicating an abnormally wide G_1 peak. A small second peak is barely seen in channel 80. The node was not histologically involved and cytogenetic analysis revealed that 16% of the cells had near diploid numerical chromosomal abnormalities. With disease relapse 9 mo later, a hyperdiploid population developed with a DI of 1.20 (panel D, arrow). The node was effaced by tumor at this time.

patients at the time of initial diagnosis. Aneuploid cells were detected by FCM in the peripheral blood and lymph nodes (8/19 patients) when light microscopic examination did not reveal tumor cells. This suggests that the presence of aneuploidy as a marker for tumor cells may be a useful diagnostic aid in staging patients with early disease when the diagnosis may be difficult to establish by other measures. The identification of malignant cells in the peripheral blood and lymph nodes of the majority of patients at diagnosis suggests that extracutaneous dissemination occurs early in CTCL.

TABLE IV
Correlation of Cytogenetic Results and FCM Results in Samples with Discrete Peaks

Sample	FCM DI*	CG modal chromosome no.‡	Aneuploid FCM	Aneuploid CG
			%	
1	1.89	90	6	8
2	1.80	82	8	10
3	1.80	82	10	15
4	1.70	88	85	42
5	1.20	53	9	13
6	1.20	50	86	40
7	1.10	49	14	9
8	1.10	49	29	11
9	0.80	37	19	47
10	0.84	43	14	10

* DI, modal channel aneuploid peak/modal channel diploid peak.
‡ CG, cytogenetics.

The FCM results provide important information relevant to natural history and prognosis. Aneuploid cells were not detected in the blood or lymph nodes of 14 patients. Malignant cells were identified by light microscopy in some of these patients. These tumor cells were, thus, diploid or near diploid and these samples demonstrate how FCM and routine histologic examination complement one another. These patients have a more indolent clinical course than patients with aneuploidy. Patients with aneuploidy (including those with a wide G_1 peak and with discrete aneuploid peaks) were more likely to have advanced disease and early relapse. Patients with discrete hyperdiploid peaks had the most aggressive disease, and all patients with the large cell CTCL histologic variant had hyperdiploid peaks. At the same time of disease relapse or

TABLE V
FCM and Cytogenetic Results before and after Mitogen Stimulation

Mitogen	Radiolabeled thymidine incorporation	FCM aneuploid	Cytogenetics aneuploid
	cpm	%	%
None	1,348	86	No mitoses
PHA	43,578	85	40
SPA	147,026	88	80

TABLE VI
Comparison of FCM Results with Peripheral Blood Cytology and Lymph Node Histology

FCM result	Cytology/histology	
	Positive (n = 45)	Negative (n = 37)
Aneuploidy (↑ CV ratio or peak)	36	13
Discrete aneuploid peak	21	3
Increased CV ratio	15	10
Normal FCM	9	24

progression, 92% of the patients had aneuploidy, 77% with hyperdiploid peaks. Four patients developed discrete hyperdiploid peaks while under study; these were always associated with relapse and transition to large cell histology with a more rapidly progressive course.

The situation is analogous to other lymphomas and leukemias. In the non-Hodgkin's lymphomas, large cell variants have the most aggressive clinical course. The small cell lymphomas (especially nodular poorly differentiated lymphocytic lymphomas) are usually indolent, and transition to a larger, more undifferentiated histology implies a poor prognosis. Our studies suggest this also occurs in CTCL. In other acute and chronic leukemias, cytogenetic changes have also been reported in association with a change to a more aggressive disease, some with changes in morphology or membrane markers (28–31).

The mechanism(s) of development of hyperdiploid clones is unknown but may include endoreduplication or cell fusion in vivo. We suggest that, initially, CTCL cells may arise with only small or no numerical chromosome abnormalities. Then, as a function of treatment or disease, aneuploid cells with discrete peaks and clones are selected and correlated with a more unfavorable prognosis.

FCM has been used to detect aneuploidy in a variety of other tumors. Aneuploidy was detected in 17% of the patients with leukemia (13), 57% with lymphoma (13), 86% with multiple myeloma (14), 45% with prostate cancer (17), 51% with bladder cancer (16), and 91% with a variety of solid tumors (14, 15). In the solid tumors, all poorly differentiated tumors have been aneuploid, whereas well-differentiated tumors may be diploid or aneuploid (16, 17), which also implies a prognostic importance for FCM results.

Cytogenetic studies have demonstrated aneuploidy in most patients with solid tumors (1, 5), but none of these FCM studies has compared cytogenetics and FCM results in patients with solid tumors. In patients with leukemia, Barlogie et al. (15) noted a good correlation between FCM and cytogenetics, but few patients had FCM abnormalities.

This report shows an excellent correlation between the DNA content detected by FCM and the chromosome number detected by cytogenetic analysis. Discrete aneuploid peaks were detected by FCM in all instances when aneuploid numerical chromosome abnormalities with numbers of 49–92 or 37–43 were present. The DI of these aneuploid peaks was always consistent with the modal chromosome number detected by cytogenetic analysis (Table III). Widened G_1 peaks (determined by CV ratio > 1.25) were present in 86% of the samples with near diploid (43-49, excluding 46 chromosomes) numerical chromosomal changes. False negative FCM results were uncommon and were restricted to instances where pseudodiploid or near diploid abnormalities were present.

Although the FCM DNA content correlated well with chromosome numbers, FCM frequently detected a larger fraction of aneuploid cells than that found by chromosome analysis. FCM analyzes more cells and is not dependent on mitogen stimulation in short-term culture as is cytogenetic analysis. The frequency of aneuploid cells detected by cytogenetic analysis after PHA stimulation in patient 6 was lower than that detected after staphylococcal protein A stimulation, suggesting PHA may not be the optimal mitogen for standard cytogenetic analyses of malignant T-cell lymphomas. Similarly, Barlogie et al. (13) found marked DNA histogram abnormalities in a small fraction of leukemic patients with normal karyotypes (possibly because only normal cells proliferated in a short-term culture). Also, Gahrton et al. (32) and Nowell et al. (31) have found that the ability to detect cytogenetic abnormalities in chronic lymphocytic leukemia is dependent on the mitogen used.

This report emphasizes that an adequate diploid standard is essential for FCM analysis. The relative DNA content of aneuploid populations (DI) and the presence of near diploid abnormalities can be detected only by using a diploid standard. The arbitrary use of a single CV (e.g., >5) is not sufficient to detect wide G_1 peaks because the CV of the standard varies from day to day as the result of differences in the instrument (e.g., laser output) and differences in sample preparation. For the study of malignant lymphomas, peripheral blood mononuclear cells are an excellent standard and, when cells can be obtained from granulocyte donors, multiple aliquots of a single sample can be frozen and retrieved whenever required.

FCM has other important advantages, including the ability to assess proliferative state by determination of the fraction of cells in G_1, S, and G_2 + M, as well as the use of aneuploidy as a marker for tumor cells when mixtures of nonmalignant and tumor cells are present. Braylan et al. (12) used FCM to study the proliferative characteristics of patients with malignant lymphomas and chronic leukemias. They found a general correlation between the percentage of cells

in S phase for a given histologic type and the expected clinical behavior. Three patients with the Sézary syndrome were studied; one of the three had >10% cells in S and had a more rapidly fatal course than the other two who had <2% cells in S. Tribukait et al. (16) reported that aneuploid bladder tumors with low proliferation were better differentiated and had more benign courses than those with high proliferation. In this study, the fraction of S cells in S phase in patients with aneuploidy was consistently higher in lymph nodes than in the peripheral blood. This is consistent with the observation that CTCL cells proliferate preferentially in lymph nodes and migrate into the circulation from these sites (33).

In addition, patients with increased S fractions appear to have a worse prognosis than those with smaller S fractions, but the numbers of patients are still small.

The FCM results in this study have enhanced our understanding of the natural history of the CTCL. Application of these techniques to other lymphomas and solid tumors may increase our understanding of these disorders as well.

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