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Clonality Assessment of Lymphoproliferative Disorders by Multiparameter Flow Cytometry of Paraffin-Embedded Tissue: An Additional Diagnostic Tool in Surgical Pathology

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A major drawback of immunohistochemical detection of monoclonality in B-cell lymphoproliferative disorders is the lack of contrast between surface-immunoglobulin staining and extracellular immunoglobulin staining. To bypass this drawback, immunophenotyping of single-cell suspensions by flow cytometry is commonly used. Although the expression of immunoglobulin light chain subtype can be quantified rapidly and reliably, the technique is hampered by the requirement of fresh unfixed material. We applied a recently developed technique for the isolation of single cells from formalin-fixed, paraffin-embedded material to measure clonality in B-cell lymphoproliferative disorders (lymphoid tissue (n = 10) and non-Hodgkin's B-cell lymphoma (n = 10). Immunocytochemistry indicated that common cell surface markers as well as the immunoglobulin light chains could be detected in the cell suspensions derived from archival material. In addition, the technique also allowed combined high-resolution DNA flow cytometric analysis. To investigate the effect of formalin fixation on cross-linking of extracellular immunoglobulins to lymphocytes, a double-immunostaining experiment for both light chain immunoglobulins (κ and λ) was performed. This experiment showed that this cross-linking was minimal (less than 2%). All cases of reactive lymphoid hyperplasia were DNA diploid and showed a polyclonal expression of immunoglobulin light chains. In contrast, in 9 of 10 non-Hodgkin's B-cell lymphomas, monoclonality was established on the basis of light chain expression, whereas only 6 of 9 cases were conclusive by immunohistochemistry. Four of the 9 cases were DNA aneuploid. One case did not show light chain expression at all by both techniques. However, this case could be classified as malignant by flow cytometric analysis because of the DNA-aneuploid nature of the B-cell subpopulation. The average S-phase fraction (SPF) of the B cells in the reactive lymphoid tissues was 3.5%. The mean SPF values for B cells in DNA-diploid cases of lymphomas was 3.0%, whereas the mean SPF of B cells in DNA-aneuploid cases was 6.1%. The presented technique is superior to immunohistochemistry for the detection of monoclonality in B-cell lymphoproliferative disorders and therefore provides a powerful tool to support the diagnosis of malignant lymphoma in routinely processed archival samples of lymphoid tissues. HUM PATHOL 31:422-427. Copyright © 2000 by W.B. Saunders Company

Key words: **light chain immunoglobulins, formalin-fixed, CD79a, immunophenotyping, CD3.**

Abbreviations: **PBS, phosphate-buffered saline; BSA, bovine serum albumin; RT, room temperature; FITC, fluorescein isothiocyanate; RPE, r-phycoerythrein; FCM, flow cytometric; CV, coefficient of variation.**

Immunophenotyping is widely used in the diagnosis and classification of lymphoproliferative disorders. For instance, establishing monoclonality in B-cell disorders plays a key diagnostic role in distinguishing neoplastic from reactive B-cell lymphocytosis.[1] These studies are usually performed on fresh frozen or formalin-fixed, paraffin-embedded tissues after antigen retrieval. Paraffin-embedded tissues are often preferred because of their superior morphology. However, a major drawback of immunohistochemistry for the detection of B-cell monoclonality is the lack of contrast between surface immunoglobulin staining and extracellular immunoglobulin staining.[2] To circumvent this drawback, phenotyping by flow cytometry has been used, offering high-speed multiparameter analysis and statistical accuracy. Several studies have compared the flexibility of immunohistochemistry and flow cytometry, in this respect.[3-5] These authors showed good correlations between both methods in most investigated cases. Quantitative analysis of the expression of light chains in the B-cell lymphocyte compartment as offered by flow cytometry seems to be superior to immunohistochemistry. It has been suggested that a κ/λ ratio of more than 3 or less than 0.5 is adequate for the diagnosis of monoclonality in the context of supporting clinical and morphological evidence of lymphoma.[6] However, this approach is limited by the availability of fresh, unfixed tissue, which often presents logistic problems in a routine setting.

In a recent study, we have described a new technique for the isolation and subsequent flow cytometric analysis of single cells from formalin-fixed, paraffin-embedded tissue.[7] The technique is based on a heat pretreatment step after dewaxing and rehydration of the tissue sections, followed by a brief trypsin digestion. Using this method, cell recovery was doubled as compared with commonly used protocols, the quality of the DNA histograms was significantly improved, and the accessibility of several antigen epitopes, including those from steroid hormone receptors[8] and membrane antigens (E-cadherin), was improved or restored. The aim of the current study was to investigate whether this protocol could be used for immunophenotyping of

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0046-8177/00/3104-0004$10.00/0
doi: 10.1053/hp.2000.6551

lymphoproliferative disorders. For this purpose, this multiparameter approach was used for lineage assignment and detection of monoclonality, next to establishment of DNA ploidy and cell cycle characteristics, in cells derived from formalin-fixed, paraffin-embedded tissues of B-cell lymphoproliferative disorders.

MATERIALS AND METHODS

Lymphoid Tissue Samples

Twenty consecutive cases of formalin-fixed, paraffin-embedded lymphoid proliferations were retrieved from the files of the Pathology Department of Atrium Medical Centre Heerlen. The cases were selected based on the diagnosis of reactive lymphoid hyperplasia ($n = 10$) or non-Hodgkin's lymphoma ($n = 10$) without paying attention to subclassifications. These paraffin-embedded tumor blocks were analyzed using multiparameter DNA flow cytometry without knowing the diagnosis. The non-Hodgkin's lymphomas had been classified according to the Revised European-American classification of lymphoid neoplasms[9] for routine diagnostic purposes. Based on the morphological findings in conjunction with immunohistochemistry, the cases of malignant B-cell non-Hodgkin's lymphomas had been classified as follicular lymphoma ($n = 4$), diffuse large B-cell lymphoma ($n = 2$), B-cell small lymphocytic lymphoma ($n = 2$), nodal marginal zone B-cell lymphoma ($n = 1$), and mantle cell lymphoma ($n = 1$). As part of routine procedure, all non-Hodgkin's lymphomas were submitted to a regional expert panel of pathologists, and diagnoses were confirmed.

Immunohistochemistry

Tissue sections (4 µm thick) were cut from the paraffin-embedded blocks, mounted on APES-coated slides, and air-dried overnight at 37°C. For immunostaining, the sections were deparaffinized in xylene and rehydrated in a descending ethanol series. Endogeneous peroxidase activity was blocked by immersion for 10 minutes in 3% hydrogen peroxide in methanol, after which the slides were rinsed in phosphate-buffered saline (PBS; pH = 7.2 to 7.4). The slides were placed in a 0.1 mol/L citrate solution (pH = 6.0) and boiled for 10 minutes in a microwave oven at 750 W. After preïncubation with 1% bovine serum albumin (BSA; Sigma, St Louis, MO, USA)/PBS (BSA/PBS-buffer) for 10 minutes, the primary antibody was applied at the appropriate dilution (Table 1) for 1 hour at room temperature (RT). After washing in PBS, the appropriately diluted secondary antibody was applied for 45 minutes at RT. In case of monoclonal primary antibodies, biotin-labeled goat anti-mouse Ig was applied, and in case of the polyclonal rabbit antiserum, biotin-labeled swine anti-rabbit Ig was used in the second step (both 1:400 diluted; DAKO A/S). After washing in PBS, the slides were incubated with streptavidin conjugated with horseradish peroxidase (1:600; DAKO A/S). After washing in PBS, peroxidase activity was detected with 3,3-diaminobenzidine (Sigma)/0.002% H_2O_2 solution. Finally, the sections were counterstained with Harris' haematoxylin, dehydrated, cleared in xylene, and finally mounted in malinol (chroma-gesellschaft; Brunschvoig Chemie, The Netherlands).

TABLE 1. Primary Antibodies Used in This Study

Antigen	Clone/ Catalog No.	Monoclonal/ Polyclonal	Dilution	References
CD3 (pan T)	A0452	Rabbit polyclonal	1:33	23
CD20 (pan B)	L26	Mouse monoclonal	1:33	24/25
CD79a (pan B)	JCB117	Mouse monoclonal	1:20	26
κ-Light chain	A0191	Rabbit polyclonal	1:500	19
λ-Light chain	A0193	Rabbit polyclonal	1:500	19
κ-Light chain-FITC	F0340	Rabbit polyclonal	1:500	19
λ-Light chain-RPE	R0436	Rabbit polyclonal	1:500	19
Negative controls:				
Mouse Ig	X0931	Mouse monoclonal	1:20	—
Rabbit Ig	X0903	Rabbit polyclonal	1:20	—
Mouse Ig-FITC	X0927	Mouse monoclonal	1:20	—
Rabbit Ig-RPE	X0930	Rabbit polyclonal	1:20	—

NOTE. All antibodies used were from DAKO A/S.

Preparation of Cell Suspensions

From each of the routinely processed paraffin-embedded tissues, 50-µm-thick sections were cut. These were deparaffinized in xylene and rehydrated in a descending ethanol series. The sections were then immersed in cold citrate solution (2 mg citric acid/mL aqua dest, pH = 6.0) and placed in a water bath at 80°C for 2 hours. After a 15-minute cooling period, sections were rinsed in PBS. The sections were digested in a solution of 1 mg trypsin (type II from porcine pancreas, Sigma, St Louis, MO) and 1 mg $CaCl_2$ in 1 mL TRIS-buffered saline (pH = 7.6) for 5 minutes at 37°C. The sample was then filtered through a 50-µm mesh nylon filter, and the cell suspension was centrifugated at 400*g*. The pellet was resuspended in 1% BSA/PBS-buffer.

Immunolabeling for Flow Cytometry

A double-label indirect fluorescence technique with fluorescein isothyocyanate (FITC)- and r-phycoerythrein (RPE)-conjugated secondary antibody reagents was used. The characteristics of the antibodies used are described in Table 1. All antibodies were derived from DAKO A/S (Glostrup, Denmark). The single-cell suspension was aliquoted into 100-µL samples ($\pm 10^7$ cells/mL). To each sample, both primary antibodies (appropriate diluted, see Table 1) were added simultaneously, that is, a polyclonal rabbit antiserum in combination with a monoclonal mouse antibody. Lineage assignment was performed by dual-color analysis using monoclonal CD79a in combination with a polyclonal antibody against CD3. For immunoglobulin light chain distribution, a dual-color analysis was performed using a monoclonal antibody directed against CD79a in combination with polyclonal anti-κ or anti-λ. After 1-hour incubation at RT, the samples were rinsed twice in PBS. Binding of primary antibodies was visualized by incubating the cell pellet simultaneously with both the secondary antibodies: goat-anti-mouse-Ig-RPE, 1:15 (DAKO A/S) and swine-anti-rabbit-Ig-FITC, 1:10 (DAKO A/S). After incubation for 1 hour at RT, the samples were rinsed twice in PBS and finally counterstained with propidium iodide (1.0 µg/mL PI, Sigma) in PBS (pH 7.4) containing 0.1 mg/mL RNAse (Serva, Heidelberg, Germany). The samples were allowed to stand for 15 minutes on ice in the dark before flow cytometric (FCM) analysis.

To investigate the effect of formalin fixation on cross-linking of serum immunoglobulins to lymphocytes, single cells of a non-Hodgkin's lymphoma (case 2) were simultaneously incubated with FITC-conjugated polyclonal κ- and RPE-conjugated polyclonal λ-antibody at the same dilutions as the nonconjugated ones.

Multiparameter Flow Cytometry

All samples were analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Cells were excited with

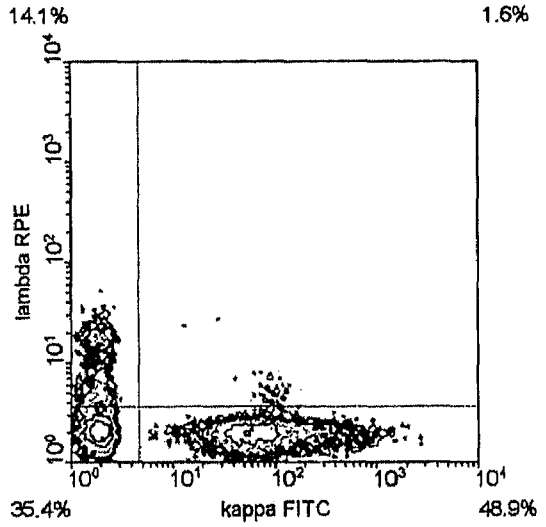

FIGURE 1. Double labeling of a single cell suspension of a non-Hodgkin's lymphoma, with directly labeled κ- and λ-antibodies, showing the mutual exclusiveness of both antibody reactions. This indicates that formalin fixation does not cross-link the extracellular immunoglobulins to the cell surface.

a single 488-nm argon laser. FITC fluorescence was detected through a 515 to 545-nm BP filter, RPE-fluorescence through a 572 to 588-nm BP filter, and PI-fluorescence through a 572 to 588-nm BP filter. Electronic gating was used to exclude doublets and cellular debris. At least 20,000 events were collected for each sample. FITC and RPE signals were recorded as logarithmic amplified data, and the PI signals were recorded as linear amplified data. The following settings were used: 450 V, 380 V, and 412 V on photomultiplier tubes for FL1 (FITC), FL2 (PE), and FL3 (PI), respectively. Compensation for (FL1-%FL2), (FL2-%FL1), (FL2-%FL3), and (FL3-%FL2) was 0.9%, 23.5%, 12.6%, and 5.6%, respectively. Data analysis was performed using Lysys II software (Becton Dickinson). A light chain ratio was calculated by dividing the percentage of CD79a-positive cells staining exclusively with κ by the percentage of cells staining exclusively with λ. A κ/λ ratio higher than 3 or lower than 0.5 is considered indicative for monoclonality.[6] Cell cycle analysis was performed using the ModFit LT 2.0 software (Verity Software House, Inc, ME). The following data were collected: (1) the percentage of the CD79a- and CD3-positive lymphocytes; (2) the percentage of κ- and λ-light chain expressing CD79a-positive lymphocytes; (3) the DNA index of the tumor cells and the coefficient of variation (CV) of the first G_0/G_1 peak; and (4) the S-phase fraction of the CD79a-positive population.

RESULTS

Microscopic examination of the fluorescently labeled cell suspension showed that these suspensions contained predominantly intact lymphocytes. Examination of the immunostained single cells by fluorescence microscopy indicated that the antigenic epitopes for CD3, CD20, and CD79a were indeed retrieved on the cell surface. For CD79a as well as for the κ and λ light chains, a membraneous or a cytoplasmic staining pattern was observed.

Examination of the double-staining experiment to test the effect of formalin fixation on cross-linking of extracellular immunoglobulins to the surface of lymphocytes showed that the population that is positive for κ- as well as λ-light chain immunoglobulins is only 1.6% (Fig 1).

The epitopes recognized by the antibodies L26 (CD20cy) and JCB117 (CD79a) are both expressed throughout B-cell differentiation and are B-cell specific. For this reason, these antibodies are used in the current study in a multiparameter FCM assay for the selection of the B lymphocytes. There was almost no difference in the number of B lymphocytes stained for L26 (data not shown) and CD79a. However, a better discrimination between immunopositive and negative cells could be made when we used CD79a as B-cell marker.

Reactive Lymphoid Hyperplasia

The percentage of reactive B lymphocytes (CD79a+) in the reactive hyperplastic lymph nodes ranged from 31% to 58%, whereas the fraction of reactive T lymphocytes (CD3+) varied from 9% to 57%. Figure 2 shows an example of such a multiparameter CD3/CD79a DNA flow cytometric analysis. The range of the κ/λ-ratio as

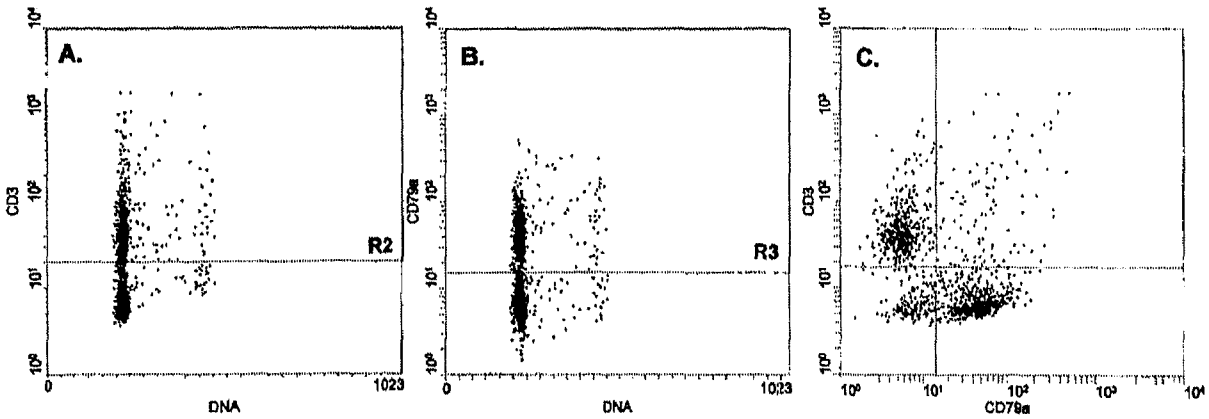

FIGURE 2. An example of a flow cytometric analysis of a reactive hyperplastic lymph node. Cells were stained in suspension with CD3, CD79a, and propidium iodide. (A) Dotplot of DNA content (x-axis) versus CD3 (y-axis). (B) Shows the signal of CD79a versus DNA. (C) CD79a versus CD3. Three cell populations can be distinguished, a population only positive for CD3 (T lymphocytes), a population only positive for CD79a (B lymphocytes), and a population negative for both.

TABLE 2. FCM Characteristics of Reactive Hyperplastic Lymphatic Tissue

Case No.	% B Cells	% T Cells	DNA-Index	CV (%)	SPF (%)	κ/λ Ratio
1	45	30	1.00	3.36	4.04	0.79
2	58	9	1.00	2.46	8.57	1.02
3	31	16	1.00	3.66	1.56	1.05
4	41	47	1.00	3.76	1.16	0.87
5	32	38	1.00	3.38	5.29	1.06
6	37	32	1.00	2.98	2.93	0.95
7	42	57	1.00	4.97	3.50	1.03
8	30	50	1.00	4.28	1.91	1.09
9	40	42	1.00	3.95	3.57	0.57
10	35	47	1.00	3.95	2.35	0.86
Mean ± SD	39 ± 8	37 ± 15	1.00	3.68 ± 0.7	3.49 ± 2.18	

NOTE. All cases were diploid.

Abbreviations: CV, coefficient of variation; SPF, S-phase fraction; MFI, mean fluorescence intensity; SD, standard deviation.

determined by FCM was 0.57 to 1.09. No case had a ratio less than 0.5 or greater than 3. DNA analysis of these suspensions showed that all cases were diploid, with an S-phase fraction ranging from 1.16% to 8.57% (mean, 3.49%). The CV of the G_0/G_1-peak in the DNA histogram ranged from 2.46% to 4.97% (mean, 3.68%; Fig 2; Table 2).

Non-Hodgkin's B-cell Lymphoma

Flow cytometric analysis of non-Hodgkin's B-cell lymphomas (Fig 3, Table 3) showed that 9 of 10 cases exhibited a monoclonal expression for 1 of the light chains. The κ/λ-ratio determined by FCM ranged from 0.14 to 15.67. Four of 10 cases showed monoclonality of the κ-light chain (eg, Fig 3), whereas the 5 other cases had a monoclonal expression of the λ-light chain. DNA analysis of the non-Hodgkin's lymphomas showed that 5 of 10 cases were aneuploid with a CV of the G_0/G_1 peak of the normal diploid cells ranging from 1.90% to 4.20% (mean, 3.30%). The S-phase fraction of the aneuploid neoplastic lymphoid tissues ranged from 1.30% to 15.88% (mean, 6.07%), whereas that of the diploid lymphomas was lower, 1.65% to 4.41% (mean, 3.03%). One case showed no expression of light chains in immunohistochemistry or flow cytometry. However, the tumor cells of this case were aneuploid. In this particular case, the diploid lymphocytes showed a poly-

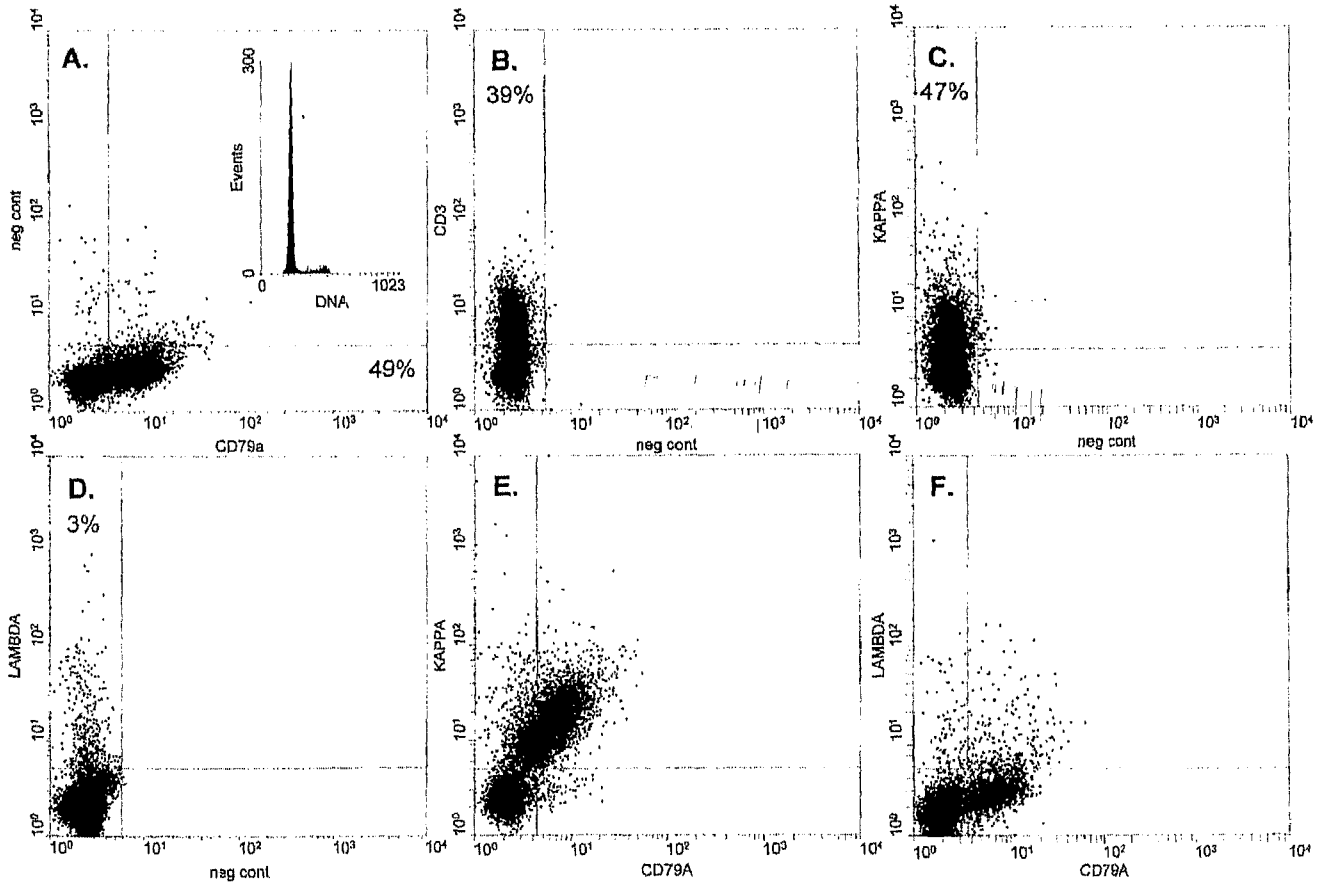

FIGURE 3. An example of a detailed multiparameter flow cytometric analysis of a non-Hodgkin's B-cell lymphoma (case 3, follicular lymphoma). The tumor cells were stained in suspension with monoclonal antibodies directed against CD79a, CD3, polyclonal anti-κ, polyclonal anti-λ, and propidium iodide. (A and B) Different single-label controls for the FITC- and RPE-fluorescence signals. (A) Cells are only stained for CD79a. (B) Cells are stained with CD3. (C and D) Expression of the light chains, with most of the cells (47%) expressing κ-light chains. (E and F) Cells are double-stained with CD79a and κ or λ. The B cells that are selected by CD79a immunostaining are almost all expressing κ, as shown in E.

TABLE 3. FCM Characteristics of the Non-Hodgkin Lymphomas

Case No.	Histological Subtype Diagnosis Malignant Lymphoma:	CD79+ Cells	CD3+ Cells	DNA Index	Ploidy	CV %	SPF %	κ/λ Ratio	Light Chain Phenotype
1	B-cell small lymphocytic	69	2	1.05	Aneuploid	1.98	1.30	4.40	κ
2	Diffuse large B cell	54	45	1.00	Diploid	3.67	3.83	3.47	κ
3	Follicular	49	39	1.00	Diploid	3.25	4.41	15.67	κ
4	Diffuse large B cell	44	47	1.21	Aneuploid	3.49	7.49	5.00	κ
5	B-cell small lymphocytic	56	18	1.05	Aneuploid	1.90	2.53	0.45	λ
6	Follicular	60	30	1.00	Diploid	3.90	2.30	0.43	λ
7	Mantle cell	88	10	1.00	Diploid	3.37	1.65	0.14	λ
8	Follicular	55	15	1.09	Aneuploid	3.58	3.13	0.31	λ
9	Follicular	80	6	1.18	Aneuploid	4.20	15.88	n.a.	No light chain
10	Nodal marginal zone B cell	72	25	1.00	Diploid	3.68	2.97	0.33	λ
Mean		63	24			3.30	4.55		
SD		14	16			0.77	4.35		

Abbreviations: L, lymphoma; CV, coefficient of variation; SPF, S-phase fraction; κ, kappa light chain; λ, lambda light chain; SD, standard deviation; NA, not applicable.

clonal expression of the κ- and λ-light chain immunoglobulins.

DISCUSSION

Using this approach, we could discriminate between reactive lymphoid hyperplasia and malignant non-Hodgkin's B-cell lymphomas. Furthermore, the clonality assessment of the light chain expression on basis of flow cytometric analysis was in 2 cases superior to immunohistochemistry. In the hematopathology of B-cell disorders, the detection of immunoglobulin expression with light chain restriction is still the most reliable criterion for the identification of B-cell non-Hodgkin's lymphomas. Until now, using standard techniques, the detection of immunoglobulins in formalin-fixed, paraffin-embedded material was less sensitive than in frozen tissue.[10-13] The malignant lymphocytes are often mixed with a reactive population of lymphocytes and plasma cells expressing both types of light chains.

An alternative technique that has been extensively used in the diagnosis of leukemia[14] and lymphomas[15-20] is the FCM analysis of the lymphoproliferative disorder. This technique offers some advantages over immunohistochemistry in terms of speed (analyzing 10,000 to 50,000 cells in a few minutes), multiple parameter analysis (including ploidy status and cell cycle phase distribution), quantitative information, and fewer problems with nonspecific background staining. With this technique, it is possible to examine subpopulations of cells discernible on the basis of size, ploidy, or immunophenotypic characteristics. Until now this technique could only be applied to fresh or frozen tissue samples, because enzymatic digestion steps, necessary for the preparation of single-cell suspensions from paraffin-embedded tissues, destroyed many epitopes, in particular those of the cell membrane–bound antigens. However, fresh or frozen tissue is not always available. Recently, we described a new protocol to prepare single-cell suspensions from paraffin-embedded tissues by a combined heating and brief enzymatic digestion step.[7] An improved DNA histogram resolution, next to a high cell recovery and a good immunoreactivity, was shown. In the current study, we applied this protocol on formalin-fixed, paraffin-embedded lymphatic tissues. Heating in citrate solution is a widely used technique for heat-induced epitope retrieval in immunohistochemistry of formalin-fixed, paraffin-embedded tissue. Many of the antibodies directed to the cell membrane–bound epitopes used in the diagnosis of lymphoproliferative disorders require such retrieval techniques. In this study, it became apparent that after heating the lymphatic tissues, an enzymatic digestion step of 5 minutes was appropriate to release enough cells to perform the FCM analysis.

An advantage of this new approach is that when using multiparameter FCM, neoplastic clones can be found by selecting cell populations by including the cell size information. These clones were otherwise obscured by reactive nonmalignant B or T lymphocytes. When studying the light chain expression in the B-cell marker–positive cells, it became apparent that the results obtained by immunohistochemistry correlated well with those obtained by FCM. However, the FCM analysis was superior for detection of light chain restriction in 2 of 10 malignant non-Hodgkin's lymphomas, because of severe background staining in the tissue sections. One case concerned a nodal marginal zone B-cell lymphoma in which neither immunohistochemistry nor PCR analysis was conclusive. However, using cell size information and multiparameter FCM, a monoclonal neoplastic clone could be detected expressing only λ-light chains. The other case was a follicular lymphoma in which the neoplastic cells had no expression of light chain immunoglobulins. However, these malignant lymphocytes were aneuploid, whereas the normal diploid reactive lymphocytes showed a clear expression of κ- as well as λ-light chains. This illustrates how this approach also may enable the selection of tumor cells in partially involved lymph nodes.

Although applied to this limited series of routinely processed lymphoproliferative disorders, multiparameter FCM analyses showed aneuploidy in 5 of the 10 investigated lymphomas (including the 3 peridiploid cases), whereas all reactive hyperplastic lymphoprolifer-

ative cases were diploid. In the literature, there is large variation in reported percentages of cases with aneuploidy found by FCM. This varies from a small number[15,16] up to 57% of the non-Hodgkin's lymphomas.[21] Overall DNA aneuploidy is encountered in 30% of non-Hodgkin's lymphomas when single-parameter DNA FCM analyses are used.[22] Conversely, when using multiparameter FCM analyses, combining a B lymphocyte and a light chain marker on fresh tissue samples, Braylan and Benson[16] found abnormal DNA contents in 80% of their cases. These results emphasize the importance of a multiparameter approach for the detection of ploidy abnormalities in non-Hodgkin's lymphomas. The studies with low numbers of aneuploidy could suffer from the rather high CVs of the G_0/G_1 peaks found in single-parameter FCM, because of admixture with cell debris. These wide CV can hide peridiploid cell populations, which are then not recognized. In the underlying study, DNA histogram resolution was optimal with a mean CV of 3.30%. In accord with Macartney et al,[22] we found no tetraploid or multiple DNA peaks in our limited series. Because the study population was selected as a learning set of benign versus malignant, one has to realize that most of these diagnosis were already made without the help of immunohistochemistry.

In conclusion, our study shows that when combining a heat pretreatment with a short trypsin digestion step for the isolation of single-cell suspensions from non-Hodgkin's lymphomas, a proper clonality assessment can be performed by FCM. As a result, the technique allows the simultaneous evaluation of cell size and 2 cell membrane–bound antigens on the same cell and provides quantitative information concerning immunophenotype, ploidy, and cell cycle characteristics of formalin-fixed, paraffin-embedded lymphatic tissue. Therefore, it can be an important aid in the diagnosis of lymphoproliferative disorders.

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