Cytogenetic and Histologic Correlations in Malignant Lymphoma

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Although a number of studies have indicated correlations between histologic subtypes of tumors and certain nonrandom chromosome changes, cytogenetic studies of lymphoma are in an early stage compared to those of leukemia. No comprehensive analysis of available data has so far been attempted in the literature either. Here we present an analysis of chromosome changes and their correlation with subtypes of lymphoma studied by conventional histology and cell surface markers, as observed in two sets of data: (1) a group of 65 karyotypically abnormal tumors sequentially ascertained and studied by us during the period January 1, 1984 to April 30, 1985, and (2) a larger data set derived by combining our data with those from two previously published series from the University of Minnesota that are comparable to our data. These combined data, which comprise the largest data set on the cytogenetics of lymphomas assembled so far, enabled a comprehensive analysis of correlation between chromosome change and tumor histology and the patterns of chromosome instability in these tumors. We found several significant associations, some previously described and others now recognized, between nonrandom chromosome gains, breaks, translocations, and deletions and histologic subtypes of tumors that characterize lymphomas. The data indicate that finding of chromosome breaks at certain sites (e.g., Bq24, 14q32, 18q21) is of diagnostic value in dealing with cases of unusual lymphoma. Furthermore, nonrandom chromosome breakage exhibited three distinct patterns that reflected three levels of etiologically relevant genetic change.

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HISTOLOGIC and immunologic studies of malignant lymphoma have led to a number of systems of classifications that have attempted to define subgroups based on biological relatedness or clinical significance.\(^1\)\(^-\)\(^4\) Recent molecular studies of Burkitt’s lymphoma and chronic myelogenous leukemia have shown that chromosome translocations play an important role in the development of neoplasia by bringing about deregulation of the genes involved in rearrangements.\(^5\)\(^-\)\(^6\) Cytogenetic studies of leukemia further have established that specific chromosome abnormalities are diagnostic and prognostic indicators.\(^7\)\(^-\)\(^8\) The clinical significance of cytogenic changes in lymphomas is less clear\(^9\)\(^-\)\(^12\) compared to leukemias where chromosome analysis is now performed as part of the clinical evaluation of patients. A number of recent studies however have demonstrated that recurring translocations t(14;18)(q32;q21) and t(8;14)(q24;q32) and the recurring deletion del(6q) are associated with certain histologic subtypes of lymphomas.\(^13\)\(^-\)\(^16\)

We have been conducting a prospective study of correlations between chromosome changes, histology, and immunologic markers in tumors from all patients with a presumptive diagnosis of lymphoma seen by the Hematology/Lymphoma Service of Memorial Sloan-Kettering Cancer Center (MSKCC) since January 1, 1984. Although, as mentioned above, previous studies have addressed translocations and deletions associated with non-Hodgkin’s lymphoma (NHL), a comprehensive review of cytogenetic abnormalities associated with lymphoma has been lacking. By utilizing a combination of our data with that reported by two groups from the University of Minnesota,\(^1\)\(^-\)\(^4\)\(^-\)\(^16\) each of which included more than 50 karyotypically abnormal tumors classified by the Working Formulation (WF; Table 1), we have been able to assemble the largest data set examined so far and undertake such a review. Our analysis confirms the previously reported associations as well as establishes new correlations. In addition, we have been able to define classes of chromosome instability in lymphoma that indicate specific mechanisms of gene perturbation that are of possible significance in lymphoma development.

MATERIALS AND METHODS

Patient population and tumor material. During the study period we have analyzed 110 histologically confirmed lymphomas removed from 110 patients whose ages ranged from 7 to 80 years (mean 50 years). Of these, 58 were males and 52 were females. In 79 patients the tumor was studied at primary diagnosis, while in 25 it was studied during relapse following previous chemotherapy or radiation therapy. Previous treatment history was unknown in the remaining 6 patients. In each of three patients, two separate specimens were received yielding a total sample of 113 specimens. Involved tissues included lymph nodes in 59, lymph node and pleural fluid in one, spleen in 16, nonlymphoid tissues (fibrous tissue, muscle, lung, etc) in 22, bone marrow in 13, and ascites alone in one patient. A piece of each tumor received in Pathology was submitted for independent studies of histology, immunological markers, and cytogenetics; in addition, a small piece was frozen immediately and stored in liquid nitrogen for molecular studies. No criteria other than availability of tissue were applied in selecting patients for study. Because of this, 24 benign lymphadenopathies and 13 Hodgkin’s disease lymph nodes also were examined during this period. The data pertaining to these specimens are not discussed here. In all cases, the tissue was processed within one hour of removal from the patient. All tissues used in this study were submitted to the laboratory for diagnostic evaluation. As such, they were removed from the patients...
after obtaining informed consent for evaluation at the time of admission to Memorial Hospital.

Histology and immunological markers. Histologic preparations were made from solid tumor tissue usually fixed in B3 and stained with hematoxylin and eosin using standard methods. Lymphomas were classified according to the WF, Rappaport, Lukes-Collins, and Kiel systems. Bone marrow and effusion specimens were examined only to ascertain the presence of lymphoma. Bone marrow biopsies were embedded in Epon plastic and stained with Giemsa. The WF classification was followed in describing tumors in this paper in order to facilitate comparison of our data with those from other published series. In the text we have referred to the different lymphoma subsets by their abbreviations as indicated in Table 1. Cell surface phenotypes were studied in cell suspensions of solid tumors (lymph node, spleen, soft tissues) while surface as well as cytoplasmic phenotypes were studied on bone marrow tumors. The methods used for these studies included study of conventional markers for lymphoid cell subpopulations (IgG-FC, C receptors, heavy and light chain immunoglobulins, and rosetting with sheep and mouse red blood cells). In the case of tumors (lymph node, spleen, soft tissues) as well as typing with a panel of monoclonal antibodies (OK-T1, OK-T3, OK-T4, OK-T6, OK-T11, i-S, OK-Ia, B1, B2, and OK-M1).

Cytogenetics. The tumor specimen was minced into a single-cell suspension immediately upon receipt in the laboratory under sterile conditions in prewarmed RPMI 1640 medium. The cells were pelleted by centrifugation at 400 rpm for ten minutes, and resuspended in the same medium supplemented with 20% heat-inactivated fetal calf serum, 1% penicillin-streptomycin, and 1% L-glutamine. Cell viability was determined by trypan blue exclusion, and the cell concentration was adjusted to approximately 10^6 living cells/mL. A direct harvest was made from one 10 mL cell suspension after exposure to 0.05 μg/mL colcemid for 1/2 hour at 37°C. A second 10 mL cell suspension was incubated at 37°C overnight with 0.025 μg/mL colcemid and harvested, and a third 10 mL cell suspension was incubated overnight at 37°C, then exposed to 0.05 μg/mL colcemid for one hour and harvested. Cell harvesting was done following procedures used for chromosome preparations from blood lymphocytes. Quinacrine banding was employed throughout in karyotyping. For each patient with a successful culture, 15 to 30 cells were analyzed under the microscope for changes in chromosome number and structure. This was followed by analysis of photographic prints of at least five cells in the case of tumors with a single abnormal clone and ten cells in the case of tumors with more than one abnormal clone; three formal karyotypes were prepared for each clone. The karyotypes were described according to ISCN. The detailed tabulation of tumor histology, cell surface markers, and karyotypes of all tumors studied by us is not published here but is available upon request from R.S.K. Chaganti.

Statistical analysis of data. We enumerated all identified chromosome abnormalities (gains and losses of individual chromosomes, translocations and deletions, and breakpoints of structural alterations) from all tumor karyotypes from our data as well as from the combined data (CD). We tested for the nond randomness of numerical and structural chromosome changes using the computer program for statistical analysis of chromosome changes written by Brodeur et al. This program tests the significance of observed deviation from the expected on two assumptions: (1) in the generation of numerical alterations all chromosomes have the same probability of undergoing nondisjunction, and (2) in the generation of structural alterations the frequency of chromosome breaks occurs proportional to the length of the chromosome arm. Conventional 2 x 2 chi-square analysis was employed to compare the frequency of occurrence of chromosome abnormalities between different histologic subtypes. The latter method has been used by Levine et al in their analysis of chromosome changes in lymphomas.

RESULTS

Histologic classification of part of the same tissue specimen that was used for cytogenetic and immunological marker studies was accomplished in 98 cases that presented as solid tumors. Cytogenetic analysis was performed on bone marrow and ascites specimens in only 14 cases. These were considered unclassified lymphomas for the purpose of this study. Cell surface markers established B cell type in 74 (86%) tumors, T cell type in four (4.7%), and null cell type in six (7%); the results were inconclusive in two (2.3%) cases. Marker studies were not performed in the remaining 27 tumors. IgM was expressed by 45 (60.1%) and IgG by 16 (21.6%) B cell tumors. More than one heavy chain was expressed in seven tumors. The kappa and lambda light chain marker both were expressed nearly equally (36 and 30 tumors, respectively). The four T cell tumors were of postthymic (or peripheral T cell type) and histologically identified as large cell or immunoblastic type. In the lymphoid cell subpopulations, no case of lymphoblastic T cell lymphoma was encountered in this study.

In our series, 77% of the specimens yielded sufficient numbers of analyzable metaphases, a cytogenetic success rate that is comparable to the 68% and 65% reported by Bloomfield et al and Kaneko et al, respectively, although Yunis et al reported a 93% to 95% success rate in their series. Among the 87 successfully karyotyped specimens, five follicular and 16 diffuse tumors (24% of total successful cases) showed only normal karyotypes. Therefore these were considered as the karyotypes of the proliferating reactive cells in the malignant tumor and were not included in the statistical analysis of the data. The remaining 66 specimens (65 tumors) showed clonal karyotypic abnormalities. A mixture of normal and clonally abnormal cells was present in five follicular and 12 diffuse tumors. More than one abnor-

| Table 1. Numbers of Tumors With Clonal Karyotypic Abnormalities in Different Histologic Subtypes of Malignant Lymphoma in CD |
|----------------|----------------|----------------|----------------|----------------|
| Reference      | Low Grade     | Intermediate Grade | High Grade   |                 |
|                | SmlY | SCC-F | Mx-F | LC-F | SCC-D | Mx-D | LC-D | SNCC | Imb | Lyb | Unclassified | Total |
| 13             | 11   | 14   | 2    | 3    | 1    | 2    | 3    | 3    | 3   | --  | --          | 42    |
| 14             | 8    | 11   | 3    | 1    | 0    | 7    | 7    | 0    | 2   | 1   | 1           | 41    |
| 15             | 16   | 21   | 4    | 9    | 1    | 10   | 18   | 6    | 3   | 2   | 2           | 92    |
| 16             | 3    | 6    | 3    | 4    | --   | --   | 7    | --   | 0   | 1   | --          | 24    |
| Present report | 3    | 10   | 4    | 3    | 4    | 1    | 19   | 3    | 3   | 1   | 14          | 86    |
| Total          | 41   | 62   | 20   | 20   | 6    | 20   | 54   | 12   | 11  | 5   | 17          | 264   |

Abbreviations: SmlY, small lymphocytic; SCC, predominantly small cleaved cell; Mx, mixed small and large cell; LC, predominantly large cell; SNCC, small noncleaved cell; Imb, large cell immunoblastic; Lyb, lymphoblastic; F, follicular; D, diffuse.
Numerical changes. Although loss of different chromosomes was noted in 14 tumors in our data, a consistent correlation with histology was not seen. With regard to gains, 29 tumors showed trisomy of 21 different autosomes (Fig 1). Trisomy of chromosomes 7, 5, 11, and 12 were most frequent, and showed strong histologic correlations. Of 19 cases of LC-D, six showed trisomy 7 and five showed trisomy 12. Of the nine cases of diffuse large cleaved cell lymphoma (LC-D), a subgroup of LC-D, three showed trisomy 11, and of the 10 cases of diffuse large noncleaved cell lymphoma (LNCC-D), another subgroup of LC-D, three showed trisomy of chromosome 5.

Among the tumors in the CD, significant deviation from expected was found in the case of trisomy of chromosomes 3 in Mx-D (5/20), 5 in Imb (3/11), 7 in LC-F (7/20) and LC-D (13/54), and 12 in SmLy (13/41) (Table 2) when tested by the method of Brodeur et al.18 When the incidence of trisomy of given chromosomes in given histologic subtypes was compared with that in the entire CD lymphoma sample using the chi-square method,19 we observed a significant excess of chromosome 8 in Mx-F (4/16) and chromosome 21 in LC-D (9/54) as did Levine et al in their series.16 We additionally found a high incidence of trisomy 4 and X in LC-D (3/54 and 8/54, respectively), 5 in Imb (3/11), and 7 in LC-F (7/20) and LC-D (13/54).

Translocations. We identified 74 translocations in the 65 tumors analyzed. Of these, 42 (56.8%) belonged to three recurrent types, namely, t(8;14)(q24;q32) (10 cases), t(11;14)(q13;q32) (three cases), and t(14;18)(q32;q21) (29 cases). The remaining 32 (43.2%) were unique translocations (Table 3). The translocations t(14;18) and t(8;14) showed strong correlation with the grade of lymphoma; the former was seen in 76.5% (13/17) of low-grade tumors, 33.3% (9/27) of intermediate-grade tumors, and 14.3% (1/7) of high-grade tumors. Of the 8 intermediate-grade diffuse tumors that showed t(14;18); five were relapse specimens from patients in whom a previous biopsy had established the diagnosis of poorly differentiated lymphocytic lymphoma. The single high-grade tumor in our series with t(14;18) was a relapse tumor previously classified as LC-D. Interestingly, this tumor also showed t(8;22)(q24;q11), which is associated with high-grade tumors. In contrast, t(8;14) was absent in low-grade tumors and was present in 18.5% (5/27) of intermediate-grade and 42.9% (3/7) of high-grade tumors. Impressive associations between specific translocations and tumor subtypes were seen in the case of t(14;18) with SCC-F (9/10) and Mx-F (4/4), and t (8;14) with SNCC (3/3). We saw only three cases of t(11;14); two of these were in LNCC-D and one was in SCC-F (Table 3).

In the CD, t(14;18) was common in low- (49.6%) and intermediate- (24.0%) grade tumors and rare in high-grade tumors (3.6%; Table 3). Its incidence in the histologic subtypes was similar to that encountered in our series. It was absent from SmLy and was seen in 71% of SCC-F and 93.8% of Mx-F. Of 16 diffuse tumors in the CD that exhibited t(14;18), nine (56.3%) were reported to have evolved from a previous nodular lymphoma.

Table 2. Summary of Recurring Cytogenetic Changes in Histologic Subtypes of Malignant Lymphoma—CD*
Table 3. Frequency of Different Translocations in Histologic Subtypes of Malignant Lymphoma: Comparison of MSKCC Data With CD

<table>
<thead>
<tr>
<th>Recurring Translocation</th>
<th>Histologic Subtype</th>
<th>MSKCC</th>
<th>CD</th>
<th>MSKCC</th>
<th>CD</th>
<th>MSKCC</th>
<th>CD</th>
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<th>CD</th>
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<th>CD</th>
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</table>

*Only translocations for which all breakpoints have been identified are included in the analysis.

In the CD, t(8;14)(q24;q32) similarly was the most common translocation in high-grade tumors (42.9%) and less so in intermediate-grade (10.0%). In the CD, t(8;14)(q24;q32) showed an 83.3% association with SNCC lymphomas. The third recurring translocation, t(11;14)(q13;q32), was absent in high-grade tumors in the CD, but was presented in low- and intermediate-grade tumors with nearly equal frequency (4.2% and 3.7%, respectively). In addition, four other translocations occurred at least twice each in the CD, but showed no correlation with histologic subtypes. These were t(3;14)(p21;q32), t(5;14)(q23;q32), t(11;14)(q23;q32), and t (14;19)(q32;q13).14,16

Chromosome 1 was one of the participants in 18/32 unique translocations in our data and was the most frequent participant in these translocations. Regions 1p31-36 and 1q21-25 exhibited clustering of translocation breaks (seven and six times, respectively). In the CD, chromosome 1 was involved in 63/413 unique translocations. Of these region 1p31-36 was involved in 21 translocations and the region 1q21-25 was involved in 17 translocations. Thus, although chromosome 1 seemed to be less frequently involved in translocation in the CD than in our series, when translocations affected chromosome 1, the frequency with which breaks clustered in the 1p31-36 and 1q21-25 was almost as high as in our series (60% vs 70%).

Deletions. We identified chromosome deletions in 29 tumors and 1p and 6q were most frequently affected (Fig 2). The histologic groups SCC-D, LC-D, and Emb were frequently affected by deletion (75%, 52.6%, and 100%, respectively). Of the four tumors with del(1p), two were LNCC-D and of the three tumors with del(3p), two were Emb.

In the CD, deletions affected all chromosomes with the exception of chromosome 21 (Fig 2). Within histologic subtypes, 6q deletions were in excess in SCC-F, LC-D, and Emb while 11q deletions were in excess in SmLy (P < 0.05).18
When the incidence of deletions in given chromosomes in given histologic subtypes was compared with that of the same deletion in the entire CD, the following significant deviations from expected were encountered: 2q in Mx-F, 3p and 5q in 1mb, 6q in LC-D, 8p and 20q in SCC-D, and 11q in SmLy (P < 0.05) (Table 2).

Chromosome instability. We identified 270 breaks associated with specific as well as nonspecific rearrangements in our series (Fig 3). In the follicular tumors 66.7% of breaks were confined to chromosome 1, 14, and 18 while in the diffuse tumors 37.9% of breaks affected these three chromosomes. Chromosome 1 showed 15.2% of all breaks with clustering in regions lp3l-36 (12/14) and iq2l-24 (18/26). The following histologic subtypes exhibited recurrent chromosome 1 breaks: SCC-F (5/10), Mx-F (2/4), LC-D (7/19), and 1mb (2/3). Breaks in the 6q21-23 region recurred in LC-D (3/19). Chromosome 7 showed 10 breaks, two in the short arm and eight in the long arm. Three of the 7q breaks were in one 1mb. Chromosome 8 was most frequently affected in diffuse tumors and all breaks at 8q24 resulted in t(8;14). Breaks in chromosome 11 were clustered in the 11q13 region and were frequently seen in LC-D (5/19). Chromosome 14 showed 50 breaks (18.6% of total breaks) and 47 of them were at 14q32. Band 14q22 was affected in one SmLy and band 14q24 was affected in one 1mb and in one unclassified tumor. Chromosome 18 showed 35 breaks of which 32 were at 18q21, and all of these were involved in t(14;18). Breaks in chromosomes 19, 20, 22, X, and Y were infrequent, although all three breaks encountered in chromosome 22 were at 22q11.

In the CD, a total of 1,211 breaks were enumerated for analysis (Fig 3). In the follicular tumors, 51.4% of breaks were confined to chromosomes 1, 14, and 18 while in diffuse tumors, 34% of breaks affected these same chromosomes. Chromosome 1 showed 13.4% of all breaks with clustering in regions lp3l-36 (52/71) and iq2l-24 (36/85), thus reflecting the clustering of translocation breaks in these sites stated above. Overall, a significant excess (P < 0.05) of breaks were seen in chromosome arms lp, 1q, 6q, 14q, 18q, and 19q. Statistically significant associations (P < 0.05) were noted for breaks in chromosome 1 and lymphomas showing SCC-F and LNCC-D histology, breaks at 8q24 and SNCC lymphomas, breaks at 14q32 and SCC-F, Mx-F, and SNCC lymphomas, and breaks at 18q21 and SCC-F and Mx-F lymphomas. Additional significant correlations occurred in a number of histological subtypes and these are summarized in Table 2. Breaks in a number of chromosome arms were clustered in specific band regions, eg, lp3l-36, 1q21-32, 6q21-23, 8q24, 11q13, 14q32, 18q21, and 19q13.

DISCUSSION

Our studies presented here clearly show that recurrent cytogenetic changes characterize each of the histologic subtypes of malignant lymphoma in which more than one tumor has been studied; some of these correlations were previously established\(^1\,\text{3}^{\text{16}}\) while others are recognized here for the first time.

The data set analyzed here represents the largest group of lymphoma karyotypes assembled so far and it shows that cytogenetic findings are of diagnostic importance in NHL of B or null cell type (Table 2). While a variety of abnormalities have been identified, most have involved breaks at a limited number of bands. The finding of breaks at certain sites (eg, 8q24, 14q32, and 18q21) in a small cell neoplasm, especially one that arises in a nonlymphoid site, indicates that the lesion most probably is a lymphoma. The most important cytogenetic abnormalities in lymphomas appear to be a group of three recurring reciprocal translocations, eight trisomies, and seven deletions. Although highly significant associations between some of the abnormalities, especially the translocations, and histologic subtypes existed, the correlations were not unique and absolute, as is the case with most other tumor-associated specific chromosome changes.\(^7\,\text{8}^{\text{8}}\) Thus, t(14;18) was very strongly associated with low-grade lymphomas and t(8;14) was associated with high-grade lymphomas. In addition, t(14;18) also was strongly associated with a nodular histology.

We have shown that breaks occurred nonrandomly in a number of chromosomal regions. Such breaks appeared to assume three distinct but recurring patterns: (1) Breaks that generate the specific translocations that are associated with specific histologic subtypes, namely, 8q24, 11q32, and 18q21 (Table 3). These translocations bring about the juxtaposition of the cellular oncogenes c-myc (8q24),\(^2^{\text{9}}\) bcl-1 (11q13),\(^2^{\text{9}}\) and bcl-2 (18q21)\(^2^{\text{23}}\) with the immunoglobulin heavy chain determinant (14q32). Translocation-mediated deregulation of c-myc has been demonstrated\(^2^{\text{4}}\,\text{25}^{\text{25}}\) and it is highly likely that the other two genes are similarly deregulated by the translocations. Translocations affecting these three sites together characterized 47.3% of cytogenetically abnormal tumors in the CD. Furthermore, in several tumors in this data set they represented the sole cytogenetic abnormality suggesting that the gene deregulation that these translocations bring about plays a primary role in the development of lymphoma. (2) Breaks that generate unique translocations that are not correlated with histologic subtypes (Table 3). The best examples of this class of breaks are the chromosome 1 sites 1p34-36 and 1q22-24. These translocations never were encountered as the sole chromosome abnormality but were always seen as additional abnormalities. The cellular oncogenes c-src\(^2^{\text{6}}\) and c-ski\(^1^{\text{7}}\) have been mapped to these two sites, respectively. Perturbations affecting these sites are common not only in lymphomas but also in tumors of diverse cell types\(^2^{\text{7}}\) suggesting that deregulation of these oncogenes may be associated with nonspecific tumor progression. (3) Breaks that generate deletions of chromosomes 1p, 2q, 3p, 5q, 6q, 8p, 11q, and 20q that correlate with histologic subtypes (Table 2). Hemizygosity or homozygosity for the nondeleted chromosome followed by reduplication of the remaining chromosome has been shown to be an important genetic event in the etiology of retinoblastoma\(^2^{\text{8}}\) and Wilms' tumor.\(^2^{\text{9}}\) Some of the sites of recurring deletions seen in our analysis of lymphoma also are the sites of cellular oncogenes, for example, c-src (1p34-36) and bcl-l (11q13). Other sites of deletion that we identified may turn out to be the sites of important genes yet to be discovered. Hemizygosity-homozygosity of the sites may act as a step in lymphoma development. Thus, the cytogenetic analysis presented here...
identified for the first time distinct levels of genetic alteration associated with lymphoma development thereby providing new leads for further molecular analysis.

Histologic parameters that relate to prognosis continue to be defined and may result in revision of current classification of lymphomas. The prognostic implications of cytogenetic changes in lymphoma remain largely as unanswered questions. One immediate question relates to diffuse tumors that show t(14;18). We have established that a proportion of these had a previous nodular histology. Whether diffuse lymphomas that represent transformation from a previous nodular histology would have a better prognosis than those that lack the t(14;18) needs to be determined. As more data from prospective series such as the ones analyzed in this paper become available, further definition of the cytogenetic correlations noted here as well as recognition of new correlations can be expected.

ACKNOWLEDGMENT

We are grateful to Dr. Clara D. Bloomfield for a preprint of the article by Levine et al. that summarized the cytogenetic analysis of the series of lymphomas studied by them and to Dr. Jorge J. Yunis for providing the full karyotypes of tumors reported in 1982.

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