Cytogenetic Findings in Peripheral T-Cell Lymphomas as a Basis for Distinguishing Low-Grade and High-Grade Lymphomas

By Brigitte Schlegelberger, Annekathrin Himmler, Elisabeth Gödde, Werner Grote, Alfred C. Feller, and Karl Lennert

Cytogenetic studies on lymph node and skin biopsy specimens and peripheral blood in 104 patients with peripheral T-cell lymphomas (PTL) were compared with histopathologic diagnoses made according to the updated Kiel classification. Low-grade lymphomas presented normal metaphases more frequently than high-grade ones (P < .0001). This difference remained significant if cases with greater than 10% and greater than 50% normal metaphases in unstimulated cultures and in cultures stimulated by different mitogens were compared. On the other hand, high-grade lymphomas more often showed aberrant clones (P < .05), triploid to tetraploid clones (P < .0001), and complex clones with more than four chromosome changes (P < .01). Low-grade PTL showed consistent cytogenetic features. Clones with both inv(14)(q11q32.1) and trisomy 8q, mostly caused by [i(8q)(q10)], were found in all cases of T-cell chronic lymphocytic leukemia (T-CLL) and T-cell prolymphocytic leukemia (T-PLL). Trisomy 3 was observed only in angioimmunoblastic lymphadenopathy with dysproteinemia (AILD)-type PTL, T-zone lymphoma, and lymphoepithelioid lymphoma. Moreover, the proportion of normal metaphases in these PTL was higher than in the other low-grade PTL (P < .01). On the contrary, T-CLL, T-PLL, and cutaneous T-cell lymphomas (CTCL) showed complex clones (P < .0001), duplications in 6p (P < .01), deletions in 6q (P < .01), trisomy 8q (P < .00001), inv(14) (P < .00001), and monosomy 13 or changes of 13q14 (P < .001) more frequently than the other low-grade PTL. Trisomy 5 and +X predominated in AILD-type PTL. A cytogenetic feature characteristic of AILD-type PTL and CTCL was unrelated clones, which were found in 15% of AILD-type PTL and 17% of CTCL. The only chromosome aberration restricted to a certain high-grade PTL was t(2;5)(p23;q35) in large-cell anaplastic lymphomas. Deletions in 8q, total or partial trisomies of 7q, and monosomy 13 or changes of 13q14 turned out to be significantly more frequent in high-grade than in low-grade lymphomas (P < .01, P < .01, and P < .05, respectively). In summary, the cytogenetic findings in our series of 104 PTL enabled us to distinguish not only between low-grade and high-grade lymphomas but also between various entities of PTL. Thus, the cytogenetic findings paralleled the histopathologic diagnoses made according to the updated Kiel classification.

T HAS BEEN SHOWN that it is possible to define biologically distinct subgroups of malignancies on the basis of cytogenetic findings. To date, the diagnostic and prognostic significance of cytogenetic findings has been shown best in acute and chronic leukemias.1 This does not rule out the possibility that new cytogenetic subgroups are still being identified.2 A number of recurrent chromosome aberrations, e.g., t(8;14)(q24;q32), t(14;18)(q32;q21), or t(11;14)(q13;q32), have been found in non-Hodgkin's lymphomas.3-5 Analogous to the situation in acute and chronic leukemias, the cytogenetic findings in non-Hodgkin's lymphomas (NHL) were shown to have clinical significance.6-9 Because in Western countries B-cell lymphomas are about four times more frequent than T-cell lymphomas, the cytogenetic data base for T-cell lymphomas from Western countries is still small.10 The aim of the present study was to compare cytogenetic findings from 104 patients with peripheral T-cell lymphomas (PTL) with the histopathologic diagnoses made in accordance to the rules formulated in the updated Kiel classification.11,12 In the updated Kiel classification the T-cell lymphomas are subdivided into low- and high-grade types. All T-cell lymphomas are PTL, except for one of the high-grade types (lymphoblastic lymphoma). The low-grade lymphomas comprise five entities: chronic lymphocytic leukemia (CLL; with some variants), cerebromedullary cell type (mycosis fungoides and Sézary's syndrome), lymphoepithelioid lymphoma (Lennert's lymphoma), angioimmunoblastic (angioimmunoblastic lymphadenopathy with dysproteinemia [AILD]) lymphoma, T-zone lymphoma, and small-cell pleomorphic lymphoma. The high-grade variants include the following: pleomorphic medium-sized- and large-cell lymphoma, immunoblastic lymphoma, large-cell anaplastic lymphoma (LCAL), and lymphoblastic lymphoma. There are significant differences with regard to the cytogenetic findings not only between low-grade and high-grade PTL, but also between various entities of PTL. Thus, the cytogenetic findings parallel the histopathologic diagnoses made according to the updated Kiel classification.

MATERIALS AND METHODS

Between 1985 and 1992, 115 patients with PTL were studied in our laboratory, all by the same methods. Eleven patients were excluded because they had special diagnoses, e.g., midline granuloma, papulosus granulomatosis, or intestinal T-cell lymphoma, or because it was not possible to assign them to a definite entity of the updated Kiel classification on the material available. Thus, 104 patients were included in the present study. A total of 58 patients have been published elsewhere. They comprise 43 patients with AILD-type PTL,13-15 5 patients with T-zone lymphoma,16 7 patients with lymphoepithelioid lymphoma,17 and three patients with T-chronic lymphocytic leukemia/T-prolymphocytic leukemia.18 They are in-
Cytogenetic studies were performed on lymph node biopsy specimens cytogenetically. The diagnostic procedure included detailed diagnoses made on the same biopsy specimens that were studied cytogenetically. Phytohaemagglutinin (PHA), concanavalin A (ConA), heavy chain (IgH) gene and T-cell receptor (TCR) gene rearrangements were identified in 77 cases (74%). In 17 further cases only single-cell aberrations, but no aberrant clones, were seen. Numerical chromosome aberrations seen repeatedly were +X in 10 cases, +3 in 25 cases, +5 in 13 cases, and +7 in 12 cases. Recurrent structural aberrations were translocations or deletions involving 1p32 in 10 cases; translocations t(2;5)(p23;q35) in 2 cases of LCAL; duplications dup(5) (q23q31-32) in 3 cases; duplications dup(6) (p12p21-22) in 8 cases; interstitial deletions in the long arm of chromosome 6, mostly between 6q15 and 6q25, in 14 cases; trisomies of 8q, mostly caused by an isochromosome i(8q)(q10), in 7 cases; loss of chromosome 13, deletions or translocations of 13q14 in 12 cases; inversions inv(4)(q11q32.1) in 7 cases; as well as translocations t(6;7)(q13;q13), inversions inv(11)(p14-15q13), and translocations t(13;17)(q11-13;p11) in 2 cases each.

Comparing the cytogenetic results in low-grade and high-grade PTL (Fig 1), the proportion of normal metaphases was higher in low-grade PTL (P < .01). Eighty-three of 84 cases of low-grade PTL (99%) showed normal metaphases, whereas this was the case in only 11 of 20 cases of high-grade PTL (58%). This difference is highly significant (P < .0001).

The differences in the proportion of normal metaphases remain significant when the results from both unstimulated and stimulated cultures were compared. This is true for cases with more than 10% normal metaphases in unstimulated cultures (P < .01), with more than 50% normal metaphases in unstimulated cultures (P < .01), with more than 10% normal metaphases in stimulated cultures (P < .01), and with more than 50% normal metaphases in stimulated cultures (P < .05).

Aberrant clones were demonstrated in 58 of 84 cases of low-grade PTL (69%) and in 19 of 20 cases of high-grade PTL (95%). This is a statistically significant difference (P < .05). A frequent finding in AILD-type PTL and in cutaneous T-cell lymphomas (CTCL) was the appearance of various single-cell abnormalities, which were seen in 12 of 52 cases (23%) and in 2 of 12 cases (17%), respectively, but only in single cases of T-zone lymphoma, lymphoepithelioid lymphoma, and LCAL. This trend turned out not to be statistically significant ( \chi^2 = 3.7; P < .05 at \chi^2 = 3.8). There were no statistically significant differences between low-grade and high-grade PTL with regard to unrelated clones. However, unrelated clones were significantly more frequent in AILD-type PTL, mycosis fungoides, and Sézary's syndrome than in other low-grade PTL (P < .05). In AILD-type PTL, up to four unrelated aberrant clones were observed.

A cytogenetic feature predominantly present in high-grade PTL was polyploidy, i.e., triploidy to tetraploidy, of the aberrant clones. Forty percent of the cases of high-grade PTL showed polyploid clones, but only 2% of the cases of low-grade PTL did so. This difference is highly significant (P < .0001).

In high-grade PTL, the aberrant clones showed a higher degree of complexity. Fourteen of 20 cases of high-grade PTL (70%) contained more than four chromosome changes, but only 16 of 84 cases of low-grade PTL (19%) did so. This difference is significant (P < .01).

Of the recurrent chromosome aberrations observed (Fig 2), trisomy 3 was seen only in AILD-type PTL, T-zone lymphoma, and lymphoepithelioid lymphoma, in 35%, 40%, and 50% of the cases, respectively. Trisomy 3 was significantly more frequent in these types of PTL than in the other types of low-grade PTL (Fig 3). Moreover, trisomy 3 turned out to be significantly more frequent in low-grade than in high-grade lymphomas (P < .01). Thus, trisomy 3, which is

| Table 1. Number of Patients With Different Types of PTL Analyzed in This Study |
|---------------------------------|-----------------|
| Type of PTL                      | No. of Patients |
| T lymphocyte (T-CLL/T-PLL)       | 5               |
| Small-cell cerebriform (Mycosis fungoides/Sézary's syndrome) | 12            |
| Lymphoepithelioid                | 10              |
| Angioimmunoblastic (AILD)        | 52              |
| T-zone lymphoma                  | 5               |
| Low-grade (total)                | 84              |
| Pleomorphic medium to large cell | 15*             |
| Immunoblastic                    | 0               |
| Large-cell anaplastic (Ki 1+)    | 5               |
| High-grade (total)               | 20              |

* Including 6 cases of cutaneous pleomorphic lymphomas.
often the clone's only aberration, is specific to AILD-type PTL, T-zone lymphoma, and lymphoepithelioid lymphoma. There were no significant differences between low-grade and high-grade PTL with regard to trisomy 5 and gain of an X-chromosome. However, if the frequencies of +5 and +X in AILD were compared with the other low-grade PTL, these chromosome aberrations were significantly more frequent in AILD (P < .05). When AILD-type PTL, lymphoepithelioid lymphoma (Lennert's lymphoma), and T-zone lymphoma were compared with the other low-grade PTL, mycosis fungoides, Sézary's syndrome, T-cell CLL (T-CLL) and T-cell prolymphocytic leukemia (T-PLL), it turned out that there were highly significant differences with regard to the proportion of normal metaphases in both unstimulated and stimulated cultures. AILD-type PTL, Lennert's lymphoma, and T-zone lymphomas showed significantly more cases with more than 10% normal metaphases in unstimulated cultures (P < .0001) or more than 50% normal metaphases in stimulated cultures (P < .0001). On the other hand, complex clones (P < .0001), duplications of part of 6p (P < .01), deletions of part of 6q (P < .01), isochromosome i(8q)(q10) (P < .0001), inversion inv(14) (P < .0001), and monosomy 13 or changes of 13q14 (P < .001) were significantly more frequent in low-grade CTCL, T-CLL, and T-PLL (Fig 3). A consistent chromosome finding in all 5 cases of T-CLL/T-PLL studied were clones with both inversion inv(14)(q11q32.1) and trisomy 8q, mostly caused by an isochromosome i(8q)(q10). This constellation was never found in the other types of PTL, although 5 cases of CTCL and 2 cases of high-grade pleomorphic T-cell lymphoma
showed either i(8q)(q10), inv(14) or t(14;14)(q11;q32.1), which is closely related to inv(14).

The only specific structural chromosome aberration in high-grade PTL was translocation t(2;5)(p23;q35). It was seen only in 2 of 5 cases of LCAL. Structural aberrations involving 1p32 were found in 20%, duplications in 6p in 15%, deletions in 6q in 35%, trisomies of 7q in 30%, t(8q)(q10) in 10%, t(14;14) in 5%, and -13/changes of 13q in 26% of the cases of high-grade PTL. All deletions in 6q involved band 6q21 and all trisomies of 7q led to an amplification of band 7q21. Deletions in 13q were interstitial deletions, with the shortest common segment being 13q14; the same band was involved in three translocations. There were no significant differences between high-grade and low-grade PTL regarding translocations and deletions in 1p32 or duplications in the short arm of chromosome 6, isochromosomes i(8q)(q10), or inv(14)/t(14;14). However, deletions of 6q, trisomies 7q, and monosomy 13 or changes of 13q14 were significantly more frequent in high-grade than in low-grade PTL (P < .01 for 6q- and +7q, P < .05 for 13/13q-; Fig 2).

DISCUSSION

The present study provided clear evidence that cytogenetic findings in low-grade PTL differ significantly from those in high-grade PTL. Certain specific chromosome aberrations, such as translocation t(2;5)(p23;q35) or the simultaneous presence of inversion inv(14)(q11q32.1) and trisomy 8q are restricted to certain pathologic entities, i.e., LCAL or T-CLL/T-PLL. A significantly higher proportion of normal metaphases was found in low-grade than in high-grade PTL, whereas polyploidy and complexity of the aberrant clones and certain chromosome aberrations were seen significantly more often in high-grade than in low-grade
PTL. Thus, cytogenetic findings in PTL can be helpful in defining a detailed diagnosis according to the updated Kiel classification.

In the present study, 95% of the cases of high-grade PTL and 69% of the cases of low-grade PTL showed an aberrant clone. This frequency is about the same as in large-scale studies of B-cell lymphomas and clearly higher than in undifferentiated T-cell neoplasias, e.g., T-ALL. Significantly more normal metaphases were found in low-grade PTL than in high-grade PTL. This difference was seen in both unstimulated and in stimulated cultures. The prognostic significance of the presence and the proportion of normal metaphases has been shown in large-scale studies on acute lymphocytic and nonlymphocytic leukemias and myelodysplasias as well as in B-CLL. Levine et al stated that the presence of normal metaphases in patients with NHL was associated with a higher complete remission rate and longer survival, whereas Schouten et al and Offit et al did not find such a correlation. Therefore, it does not seem surprising to find a higher proportion of normal metaphases in low-grade PTL than in high-grade PTL. However, an uncommon cytogenetic finding is the presence of unrelated nonclonal and clonal chromosome abnormalities. The trend toward a higher frequency of unrelated nonclonal chromosome aberrations in AILD-type PTL and in low-grade CTCL could not be confirmed by statistical analysis; however, unrelated clones were significantly more frequent in these entities than in the other low-grade PTL. The presence of unrelated chromosome abnormalities in AILD-type PTL was also described by Kaneko et al., Johnson et al and Shapiro et al found more CTCL with single-cell abnormalities than with aberrant clones. In a review of the literature, Heim and Mitelman reported that the frequency of unrelated clones in malignant lymphomas was 0.6%. In our series, the frequency of unrelated clones was 15% in AILD-type PTL and 17% in mycosis fungoides/Sézary’s syndrome. Thus, the frequency of unrelated clones in these entities far exceeds the frequency in malignant lymphomas in general.

In the present study, all 5 cases of T-CLL/T-PLL presented both inv(14) and trisomy 8q, whereas inv(14) or trisomy 8q were also seen in other types of PTL. Similar findings were reported by Zech et al. It remains to be evaluated whether PTL with inv(14) and trisomy 8q represent a distinct clinical and histopathologic entity. Moreover trisomy 3, trisomy 5, gain of an X-chromosome, and t(2;5)(q23;q35) represented chromosome abnormalities that were specific to certain histopathologic entities. Trisomy 3 is known to be a characteristic change in AILD-type PTL, T-zone lymphomas, and in lymphoepithelioid lymphomas. Trisomy 5 and a gain of the X-chromosome were repeatedly reported in AILD-type PTL. In the present study, 4 cases with duplications in the long arm of chromosome 5 were detected. So far, the molecular mechanisms of how trisomy 5 contributes to tumor development are not understood. The repeated findings of a duplication dup(5)(q23;q35) may help to define the critical region of 5q, where genes important for lymphomagenesis are located. In the critical region 5q32, a number of growth factor and growth factor receptor genes, e.g., the genes for IL-3, IL-4, IL-5, CSF2, and IL-9 have been localized. The translocation t(2;5) was first described as a characteristic chromosome abnormality in malignant histiocytosis. Meanwhile, it is regarded as characteristic of LCAL. In agreement with Offit et al, the present study includes cases of LCAL with chromosome changes other than t(2;5).

Although the present study was unable to define any chromosome aberration that was specific to high-grade pleomorphic T cell lymphomas, high-grade PTL presented characteristic cytogenetic features. These features included triploid to tetraploid chromosome numbers, complexity of aberrant clones, total or partial trisomy 7q, deletions in the long arms of chromosomes 6, and losses of chromosome 13 or structural aberrations involving 13q14. Similar characteristic cytogenetic features of high-grade PTL have been also observed in adult T-cell leukemia/lymphoma, which represents a variant form of pleomorphic T cell lymphoma. Polyplody, trisomy 7q, structural aberrations of 6p, and charges of 13q14 were also seen in a considerable number of cases of mycosis fungoides or Sézary’s syndrome. This finding may reflect the fact that cytogenetic results can be obtained most easily in advanced stages of mycosis fungoides and Sézary’s syndrome. The establishment of a polyploid clone seems to be a late event during tumor development. One patient with Sézary’s syndrome (case no. 42) presented a hypodiploid and a hypotetraploid clone. The hypodiploid line was detected in the peripheral blood and in one lymph node, the hypotetraploid clone in the skin and in another lymph node. Crossen et al reported a case of Sézary’s syndrome in which the aberrant clone had two lines with 76 and 98 to 100 chromosomes, respectively. Berger and Bernheim observed a case of Sézary’s syndrome with both a hypodiploid and a hypotetraploid clone. The hypotetraploid cells may develop from hypodiploid cells by endoreplication. This mechanism may explain the development of giant cells, so-called mycosis cells, in CTCL. Interestingly, some low-grade PTL with cytogenetic features characteristic of high-grade PTL, such as structural changes in 1p32 or 6q- markers, showed morphologic criteria for the transition to high-grade lymphoma.

The comparison of our findings with those from the literature is complicated by the use of different classification systems. According to the results of the Fifth International Workshop on Chromosomes in Leukemia Lymphoma, changes in the short arm of chromosome 1 are significantly more frequent in T-cell than in B-cell lymphomas. Mecucci et al described chromosome aberrations in the short arm of chromosome 6 as characteristic changes of T-cell lymphomas. Their series included 1 case of lymphoblastic lymphoma, 2 of "poorly differentiated lymphocytic lymphoma," and 1 of Sézary’s syndrome. Seventeen percent of the 260 lymphomas reevaluated during the Fifth International Workshop on Chromosomes in Leukemia Lymphoma had a deletion in the long arm of chromosome 6. No significant correlations between the breakpoint or the amount of deleted material and the histologic or immunologic type of NHL could be shown. Schouten et al found
6q- markers to be associated with high-grade immunoblastic lymphomas. It was suggested that a tumor suppressor gene or tumor suppressor genes on 6q are eliminated by deletions in 6q, resulting in hemizygosity of the putative tumor suppressor gene, which may be affected by point mutation or submicroscopic deletions. Barletta et al and Okada et al ruled out the hypothesis that this putative tumor suppressor gene is the c-myb gene, which had been localized to 6q22-24. To the best of our knowledge, a nonrandom involvement of 13q14 in T-cell lymphomas has not been reported. Recently, a new tumor suppressor gene distal to the well-known retinoblastoma gene has been identified. Further studies are needed to examine whether this tumor suppressor gene is involved in PTL with structural changes of 13q14. Moreover, further studies should clarify the prognostic significance of the cytogenetic findings that have been shown to be associated with low-grade or high-grade PTL in the present study.

ACKNOWLEDGMENT

We thank the clinicians who provided the samples, especially Prof W. Sterry (Ulm, Germany), Prof H. Bartels (Liebeck, Germany), and Prof R. Kuse (Hamburg, Germany). We also thank Dr H.J. Plendl for statistical analysis, Jens Deerberg for preparing graphics, and K. Dege for her help in editing the manuscript.

REFERENCES


Cytogenetic findings in peripheral T-cell lymphomas as a basis for distinguishing low-grade and high-grade lymphomas

B Schlegelberger, A Himmler, E Godde, W Grote, AC Feller and K Lennert

Updated information and services can be found at:
http://www.bloodjournal.org/content/83/2/505.full.html

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml