Molecular Analysis of Cutaneous B- and T-Cell Lymphomas

By Antonino Neri, Nicola Stefano Fracchiolla, Elena Roscetti, Silvia Garatti, Dino Trecca, Alketa Boletini, Lucia Perletti, Luca Baldini, Anna Teresa Maiolo, and Emilio Berti

Among extranodal non-Hodgkin’s lymphomas, primary cutaneous lymphomas (CLs) represent a consistent group of B- and T-cell malignancies. We investigated the arrangement of Ig and T-cell receptor (TCR) genes, together with the involvement of several oncogenes and the tumor-suppressor gene p53, in a panel of primary cutaneous B- and T-cell lymphomas (CBCLs and CTCLs). Southern blot analysis was performed to detect rearrangements of the Ig, c-myc, bcl-1, bcl-2, bcl-3, bcl-6, and the NFKB2/lyt-10 genes in 52 cases of CBCLs and of the TCR, bcl-3, and NFKB2/lyt-10 genes in 38 cases of CTCLs. Tal-1 gene deletions were analyzed in CTCLs by means of polymerase chain reaction (PCR). p53 gene mutations were assayed using PCR, single-strand conformation polymorphism analysis, and direct DNA sequencing in CBCL and CTCL cases. Clonal rearrangements of Ig genes or oncogenes were found in 25 of the 52 CBCLs. In particular, we detected rearrangements of the bcl-1 locus (2 cases), the bcl-2 gene (2 cases), the NFKB2/lyt-10 gene (2 cases), and the bcl-6 gene (1 case); interestingly, 4 of these cases showed a germline arrangement of the Ig genes. Clonal rearrangements of TCR genes were detected in 37 of the 38 CTCLs. Rearrangements of the NFKB2/lyt-10 gene were present in 2 cases and tal-1 gene deletions in 3 CTCL cases; p53 gene mutations were detected in 1 CTCL case. Overall, our data indicate that (1) clonal rearrangement of Ig genes is frequently undetectable by means of Southern blot in CBCLs (60%); (2) genetic lesions are involved in a limited but significant fraction of primary CLs showing a molecular marker of clonality (13/62; 20%); and (3) rearrangements of the bcl-1, bcl-2, or bcl-6 loci, associated with specific subsets of nodal lymphoid neoplasias, are rarely observed in CBCLs. Moreover, our results suggest that tal-1 gene deletions may play a pathogenetic role in non–acute T-cell malignancies and that, in the context of lymphoid malignancies, CLs may represent a favorable target for the possible oncogenic potential of the NFKB2/lyt-10 gene.

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Primary Cutaneous lymphomas (CLs) are one of the most frequent types of extranodal non-Hodgkin’s lymphomas (NHLs), accounting altogether for 25% of all NHLs. On the basis of morphologic, immunophenotypic, and more recently, genotypic criteria, CLs have been mainly classified as cutaneous T-cell lymphomas (CTCLs) and cutaneous B-cell lymphomas (CBCLs).

CTCLs are the most frequent type of CLs. Their histologic and clinical characteristics have been extensively investigated over the last 10 years, leading to the definition of a number of pathological entities, the most frequent being Mycosis Fungoides (MF), Sézary’s syndrome (SS), polymorphic T-cell lymphomas (PTLs), and anaplastic large-cell lymphomas (ALCLs). The vast majority of CTCLs express a predominant helper phenotype (CD4+) of the neoplastic cells, but some specific subsets show a predominant suppressor/cytotoxic (CD8+) or γ/δ lymphoid cell proliferation has been identified. As far as prognosis is concerned, MFs generally present a slow evolution, even though 5% to 20% of cases may transform in aggressive ALCs. In addition, primary cutaneous CD30+ ALCs have a more indolent course than nodal CD30+ ALCs.

CLCs have been less characterized, because they were first considered to be pseudolymphomas or secondary localizations in patients with extracutaneous B-cell malignancies. It is only recently that a more careful clinical staging, together with immunohistochemical and genotypic analysis, has shown that most CBCLs represent de novo independent tumors. However, there is still no general agreement as to the cellular origin of CBCLs, and a germinal center as well as a marginal zone origin has been proposed. To date, the majority of CBCLs have been classified on the basis of their cytotype as follicular center-derived B-cell lymphomas and subdivided according to the Kiel classification into centroblastic (Cb), centrocytic (Cc), and centroblastic/centrocytic (CbCc) lymphomas. CBCLs share a fairly constant immunophenotype, characterized by the expression of pan-B-cell markers (CD19, CD20, and CD22) and the absence of CD5 (characteristic of the Cc lymphomas). CBCLs show the morphologic and immunohistochemical characteristics of immunocytoma (IC), typically expressing CD38 and cytoplasmic κ or λ Ig chains. Clinically, CBCLs behave as low-grade malignancies. When the disease is limited to the skin, the prognosis is good, regardless of its clinical presentation and despite its tendency to local recurrences. Furthermore, CBCLs generally have
a good response to radiation therapy, whereas systemic chemotherapy is indicated in only a minority of cases presenting widespread cutaneous lesions or systemic dissemination.26,37-39

The molecular pathogenesis of primary cutaneous lymphomas remains largely unknown. Their relatively low frequency, the need for skin biopsies, and the difficulty of obtaining suitable material for karyotypic and genotypic analysis have not yet allowed specific cytogenetic and molecular lesions to be identified. In particular, the role of known oncogenes and tumor-suppressor genes in the pathogenesis of CBCLs and CTCLs has not been extensively investigated. In this context, the absence of c-myc and bcl-2 oncogene rearrangements and the low level or absence of bcl-2 protein expression have been shown by us and others in primary CBCLs.32,40-44 On the other hand, the molecular arrangement of Ig and T-cell receptor (TCR) genes have been studied by several investigators.32,44,45,47 Interestingly, a lack of clonal Ig and TCR gene rearrangements has been reported in a variable proportion of cases diagnosed as malignant NHLs on the basis of morphologic and immunohistochemical criteria.37,49

These considerations, together with the availability of a large series of primary cutaneous B- and T-cell lymphomas, prompted us to undertake a comprehensive analysis of the pathogenetic role of a number of oncogenes, putative oncogenes, and the tumor-suppressor gene p53, as well as of the molecular arrangement of immune genes in a representative panel of primary CBCLs and CTCLs.

MATERIALS AND METHODS

Patients, histomorphology, and immunohistochemistry. Ninety cases of primary CLs (52 CBCLs and 38 CTCLs) admitted to the Clinica Dermatologica I of the University of Milan were selected for the study. Extracutaneous involvement was ruled out by careful clinical examination, total body computer tomography (CT) scan, bilateral bone marrow biopsy, and absence of extracutaneous involvement after at least 6 months of follow-up.

Table 1. Primary CBCLs and CTCLs included in the Study

<table>
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<tr>
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<tr>
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<tr>
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<tr>
<td>PTL M/L</td>
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<tr>
<td>ALCL</td>
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Table 2. c-myc and bcl-2 Gene Rearrangements and bcl-2 Protein Expression

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<tr>
<td>ALCL</td>
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Evaluating the cutaneous lesions at diagnosis and during follow-up (Table 2),27,29,31,40,46,47 (2) histomorphologic criteria of the updated Kiel classification34 on ematossilin-eosin sections, and (3) careful immunohistochemical analysis on frozen tissue sections using the alkaline phosphatase antialkaline phosphatase (APAAP) method.49

The following monoclonal antibodies (MoAbs) were used for immunohistochemical analysis (all, except where indicated, from the International Workshop and Conference on Human Leukocyte Differentiation Antigens, III and IV):48,52: anti-CD5, -CD10, -CD20, -CD21, -CD22, -CD23, -CD32, -CD79a, -CD45RA, and -DR-1, and anti-CD3, -CD4, -CD5, -CD7, -CD8, -CD25, -CD30, -CD45RO, -TCR-1 (T-Cell Science, Cambridge, MA), and -B1 (T-Cell Science) for CTCLs. bcl-2 protein expression was investigated by means of anti- bcl-2 MoAb clone 124 (Dako, Glostrup, Denmark) on both formalin-fixed, paraffin-embedded and frozen tissue sections using the APAAP method.50 Primary CBCLs were represented by 25 cases of CbCc diffuse lymphomas, 10 cases of CbCc follicular/diffuse (F/D) lymphomas, 12 cases of Cb lymphomas, 2 cases of lymphocytic lymphomas (Ly), and 3 cases of lymphoplasmocytoid lymphomas (LPC; Table 1). Furthermore, we included 1 case of the secondary skin localization of a nodal Cc NHL and 2 cases of the secondary skin localizations of primary nodal follicular NHLs (data not shown). Primary CTCLs were represented by 24 cases of MF, 6 cases of small/medium cell PTLs (PTL S/M), 5 cases of medium/large cell PTLs (PTL M/L), and 3 cases of ALCLs (Table 1). Molecular characterization was performed in all 90 cases of primary CLs.

DNA preparation and Southern blot analysis. Frozen pathologic samples were minced on dry ice and the DNA was purified by proteinase K digestion, phenol-chloroform extraction and ethanol precipitation.53 For Southern blot analysis, 10 µg of DNA from each sample was digested to completion with the BamHI and EcoRI restriction enzymes, size fractionated in 0.8% agarose gel, denatured, neutralized, and transferred to nylon membranes (Hybond-N+; Amersham, Amersham, UK). The blots were hybridized to random primed9-11 labeled probes according to the manufacturer’s specifications, washed in 0.5% SSC/1% sodium dodecyl sulfate (SDS) for 1 hour at 60°C, and autoradiographed at -80°C. The rearrangements of the IgH locus were analyzed in BamHI and EcoRI digests hybridized to a 6.6-kb BamHI-HindIII DNA probe specific for the joining region of the IgH locus (JH).54 All of the cases in which a germline configuration of the IgH locus was detected were further investigated by the analysis with the Jκ probe55 specific for the joining region of the κ light chain locus. Cκ,56 Cκ,56 and Cy probe56,66 probes, specific for the constant regions of the IgH locus and CA probe56,57 specific for the constant region of the λ light chain locus, were also used to analyze cases EB312, EB339, EB585, and EB773 (see Results). The rearrangements of the TCR locus were analyzed in BamHI and EcoRI digests hybridized to the constant region of the TCR γ chain locus59 and to the joining region of the TCR γ chain locus60 and the arrangement of the c-myc locus was investigated by means of hybridization of BamHI-, EcoRI-, and HindIII-digested DNAs to the human MC413RC probe56, representative of the third exon of the c-myc gene. The bcl-2 locus was analyzed by the hybridization of BamHI- and EcoRI-digested DNA to the pF1 probe, which is specific for the major breakpoint region (MBR),60 and to the pFL2 probe, specific for the minor cluster region (MCR) of the bcl-2 gene.53

Rearrangements of the bcl-1 locus were investigated by the hybridization of BamHI and EcoRI digests to the probe pF1.53 The bcl-6 locus was investigated by the hybridization of BamHI and Xba1 DNA digests to the bcl-6 genomic probe (40-kb Sac I fragment).56

Rearrangements of the NFkB2/lyt-10 locus were investigated by the hybridization of BamHI and EcoRI digests to the probes PvsH 1.4,
### Table 2. Clinicopathologic, Immunohistochemical, and Genotypic Features of CBCCLs Included in the Study

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<th>Follow-up (mo)</th>
<th>Treatment</th>
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<th>bcl-1</th>
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We characterized at the molecular level 52 primary CBCLs and 38 primary CTCLs, diagnosed on the basis of clinical evaluation, histopathologic criteria of the updated clinical classification and staging system of the National Cancer Institute, and more recently analyzed by hybridization to BAC or t (3). The molecular analysis included the study of the IgH loci, TCR beta loci, and p53 tumor-suppressor gene. 

RESULTS

We characterized at the molecular level 52 primary CBCLs and 38 primary CTCLs, diagnosed on the basis of clinical evaluation, histopathologic criteria of the updated clinical classification and staging system of the National Cancer Institute, and more recently analyzed by hybridization to BAC or t (3). The molecular analysis included the study of the IgH loci, TCR beta loci, and p53 tumor-suppressor gene. 

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Table 3. Clinicopathologic, Immunohistochemical, and Genotypic Features of CTCLs Included in the Study

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Abbreviations: S/M, small/medium cells; M/L, medium/large cells; NT, no therapy; LR, local radiotherapy; CT, systemic polichemotherapy; BMT, bone marrow transplantation; IFN, interferon α; PUVA, psoralen plus UVA phototherapy with; UVB, phototherapy (UVB); S, systemic steroid therapy; SK, skin; PBL, peripheral blood; BM, bone marrow; LN, lymph node; CR, complete remission; PR, partial remission; LF, lost to follow-up; NR, no relapse; B-NHL, B-cell non-Hodgkin's lymphoma; D, died of lymphoma; - , <10% cells positive; +, from 10% to 30% cells positive; ++, from 30% to 70% cells positive; ++++, >70% cells positive; ND, not done; G, germline; R, rearranged; M, mutated.

* CTCLs with TCR genes rearrangement.
† After withdrawal of diphenylhydantoin.
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Fig 1. Southern blot analysis of IgH gene rearrangements in CBCLs. DNAs were digested with BamHI restriction enzyme and hybridized with the JH probe (see Materials and Methods). The germline band is indicated by a dash and its size is given in kilobases. All of the cases shown presented a clonal rearrangement of one or two IgH alleles. C, human placental DNA.

In these 4 cases, the possible occurrence of rearrangements affecting other regions of the IgH locus (namely Cμ, Cα, and Cγ) and the λ light chain gene was further investigated, and a germline arrangement of the analyzed loci was observed (data not shown). The clinicopathologic, immunohistochemical, and molecular characteristics of the 52 primary CBCLs with or without IgH locus or oncogene rearrangements are shown in Table 2. The clinicopathologic features of all of the primary CBCLs showing an oncogene rearrangement are illustrated in Fig 3.

The bcl-2 gene located on chromosome 18q21 is involved in reciprocal translocations affecting the IgH locus on chromosome 14q32 that have been shown to be specifically associated with follicular NHLs. In our series, rearrangement of the bcl-2 gene took place at the MBR region, where the majority of breakpoints described so far are clustered. The rearranged cases (EB773 and EB834) were a CbCc and a Cb primary CBCLs, both presenting a diffuse pattern of tumoral infiltration (Figs 2 and 3 and Table 2) without CD10 antigen expression (Table 2). Case EB773 did not show any Ig rearrangement, at the level of either the heavy or the light chain loci, whereas case EB834 showed no comigration of the bcl-2 and IgH rearranged alleles (Fig 2). As shown by immunohistochemistry, both cases presented relatively high levels of bcl-2 protein expression (Table 2). However, the expression of bcl-2 protein was not exclusively associated with bcl-2 gene rearrangement, having been detected in approximately 48% of the 52 CBCLs studied (Table 2). This findings are in accordance with previous reports.

Although case EB834 developed nodal involvement and underwent systemic chemotherapy, Rearrangements affecting the MBR region of the bcl-2 gene were also detected in cases EB336

Fig 2. Southern blot analysis of primary CBCLs with oncogene rearrangements. For each case, the Ig heavy chain gene and oncogene analyses are shown. The restriction enzymes used are indicated. Germline bands are indicated by dashes. With the exception of cases EB341 and EB834, all of the cases show a germline configuration of the JH locus. Cases EB773 and EB834 show a bcl-2 gene rearrangement affecting the major breakpoint region with the PFL-1 probe; and cases EB339 and EB341 show a bcl-1 gene rearrangement with the MTC and p94 probes, respectively. Case EB585 shows two different rearranged fragments with the NFKB-2/lyt-10 probes P/H 1.4 and P/P 1.1, representative of the 5' and 3' region of the gene, respectively. Case EB312 showed a bcl-6 gene rearrangement with the Sac 4.0 genomic probe.

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Fig 3. Histologic pictures of the primary CBCLs with oncogene rearrangements. Case EB773: CbCc lymphoma. Detail of the cytologic picture with several medium-size cleaved centrocytoid cells and reactive T cells. Lower magnification, a band-like infiltrate in the superficial dermis. Case EB834: Cb lymphoma. Large centroblastoid cells with atypical and pleomorphic nuclei. Lower magnification, a nodular infiltrate in the whole dermis. Case EB339: Cb lymphoma. Clusters of large cells. Lower magnification, the neoplastic area shows a periadnexal and perivascular lymphoid infiltrate. Case EB341: CbCc F/D lymphoma. Neoplastic centrocytoid cells near a germinal center area. Lower magnification, follicular and diffuse infiltrate sparing the superficial dermis (Grenz Zone). Case EB312: Lymphocytic lymphoma. Round infiltrate with few cleaved cells and several lymphoblasts. Lower magnification, nodular diffuse infiltrate with sparing of the superficial dermis (Grenz Zone).

and EB940, which are represented by cutaneous localizations of nodal follicular NHLs typically expressing CD10 antigen (data not shown); in both cases it was possible to demonstrate comigration of the bcl-2 and IgH rearranged alleles (data not shown). Both cases presented bcl-2 protein expression as shown by immunohistochemistry (data not shown).

The reciprocal translocation t(11;14)(11q13;14q32), affecting the bcl-1 and IgH loci, represents the specific genetic lesion of Cc NHLs. The majority of the bcl-1 rearrangements involve the MTC region, but sporadic breakpoints have been described to occur also outside this cluster, mainly affecting sequences located \(\approx 20\) kb upstream from the MTC region. In our panel, we were able to demonstrate rearrangements of the bcl-1 locus involving both of these regions. In particular, case EB339 showed the breakpoint within the MTC region; in case EB341, the rearrangement occurred within the region located \(5'\) to the MTC, as indicated by the pattern of hybridization to the p94 probe. In both of these cases, there was no evidence of any Ig genes rearrangement (IgH and \(\kappa\) light chain loci; Fig 2, data not shown, and Table 2). Neither of the cases presented the typical histologic and immunophenotypical pattern of Cc NHLs. Case EB341 morphologically resembled nodal NHLs originating from the germinal center, whereas case EB339 showed the histologic picture characterizing nodal high-grade large-cell NHLs; furthermore, neither of the cases expressed CD5 antigen (Table 2 and Fig 3). Clinically, both cases presented the chronic, slowly progressive course of CBCLs, remaining limited to the skin (Table 2). A rearrangement affecting the MTC region of the bcl-1 gene was also observed in case EB1241, a cutaneous localization of a primitive nodal Cc NHL, typically expressing CD5 antigen (data not shown). In this case, the comigration of bcl-1 and IgH rearranged alleles was demonstrable (data not shown).

A number of different cytogenetic abnormalities involving chromosome band 3q27 have been consistently associated with NHLs, and the corresponding proto-oncogene on band 3q27 has recently been identified as a member of the zinc-finger transcription factor family and variously called bcl-6 or LAZ3. In our series, rearrangement of the bcl-6 gene was detected in 1 case of primary diffuse lymphocytic CL (case EB312) that did not show any evidence of rearrangements affecting the IgH or the \(\kappa\) and \(\lambda\) light chain loci (Figs 2 and 3 and data not shown). The patient rapidly developed nodal involvement and underwent systemic chemotherapy (Table 2).

NFkB2/llyt-10 rearrangements were detected in cases EB159 and EB585. Case EB159, a CbCc primary CBCL, has been previously described at the molecular level in the context of an NFKB2/llyt-10 gene involvement analysis of a large panel of lymphoid malignancies. In case EB585, a CbCc F/D primary CL (Table 2 and Fig 3), NFKB2/llyt-10 gene analysis identified a rearranged band in BamHI and EcoRI digests after hybridization to the PvuII 1.4 probe, representative of the \(5'\) region of the gene. Rehybridization of the same filter with the P/P 1.1 probe, representative of the \(3'\) region of the gene, showed a novel rearranged restriction fragment, suggesting the occurrence of a chromosomal translocation generating two reciprocal recombination products.
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Fig 4. PCR analysis of SlLtal-1 fusion regions. (A) Ethidium bromide-stained agarose gel of PCR-amplified DNA fragments from the positive cases EB600, EB604, and EB639 and the negative case EB981. MW, 6X/HaeIII molecular weight markers; CEM, CEM cell line (tal-1 deletion positive control); N, placental DNA (tal-1 deletion negative control). The separately amplified fragments from the tal-1 gene (see Materials and Methods) (571 bp) and SlLtal-1 fusion gene (350 bp) of each case were contemporary loaded; molecular weights are indicated. (B) Southern blot analysis: hybridization with the SIL oligonucleotide probe confirmed the specificity of the PCR analysis of tal-1 deletion.

(Fig 2). In this case it was also not possible to show any rearrangement affecting the IgH locus (Fig 2) or the κ and λ light chain loci (data not shown). The clinical course of the 2 patients was different: case EB159 had an aggressive evolution, with the development of mixed cryoglobulinemia and nodal involvement 6 months after diagnosis that required systemic chemotherapy; case EB585 had a slow evolution, with the regression of the cutaneous lesions after radiotherapy.

No rearrangements of the c-myc and bcl-3 genes were detected in any of the samples examined by means of Southern blot (data not shown).

Analysis of the p53 gene by PCR-SSCP did not show any alterations in the normal migrating electrophoretic pattern suggestive of mutations in any of the cases (data not shown).

Analysis of CTCLs. Immunogenotypic analysis detected clonal rearrangements involving TCR β and/or γ chain genes in 37 of the 38 (=97%) CTCLs included in the study (Tables 1 and 3); the remaining ALCL case showed a germline arrangement of these two loci. These findings, together with the absence of CD3 surface antigen and the expression of CD30 antigen, suggest that the neoplastic cells of this ALCL case could be frozen at a prethymic stage of differentiation (Table 3).

No rearrangements of the bcl-3 gene, as assessed by Southern blot analysis using a full-length cDNA probe, were found in any of the examined samples (data not shown).

Two main mechanisms are responsible for tal-1 gene rearrangements: chromosomal translocation t(1;14)(p32;q11) involving the TCRδ locus and deletions affecting its 5’ flanking region, causing the juxtaposition of tal-1 and SIL genes. We investigated the presence of deletions affecting the 5’ region of the tal-1 gene by using a PCR-based approach that took advantage of two sets of primers identifying the majority of the reported deletions and were able to detect the fusion of these two genes at the genomic level in 3 of 38 patients (=8%; cases EB600, EB604, and EB639), represented by 2 MFs and 1 PTL S/M CD8⁺ lymphoma, respectively (Fig 4 and Table 3). From the clinical point of view, these patients presented an unusually aggressive and rapidly progressive course of the disease, with a poor response to systemic polychemotherapy. In particular, case EB639 rapidly progressed from PTL to immunoblastic lymphoma, dying 6 months after diagnosis with massive skin involvement, without any apparent systemic spreading of the disease.

The NFKB2/lyt-10 rearrangements found in 2 cases of tumoral stage MFs (cases EB599 and EB625) have been previously described. Both of these cases showed an atypical aggressive clinical course, requiring systemic polychemotherapy: case EB599 presented a rapidly fatal course and case EB625 developed peripheral blood involvement 5 years

Fig 5. DNA direct sequencing analysis of p53 mutations in CLs. The fragment corresponding to exon 5 of case EB597 was amplified as described in the Materials and Methods. The 5’ primer used for PCR amplification was also used as sequencing primer. The nucleotide sequence corresponding to human placenta DNA (Normal) is shown and the mutated base pair is indicated by an arrow.
after diagnosis, dying 2 years later as a result of the consequences of the systemic spreading of the disease.

Analysis of the p53 gene by PCR-SSCP showed the occurrence of a mutation only in 1 case of tumor stage MF (case EB597; data not shown). The mutation occurred at codon 163 of the p53 gene and was represented by a missense point mutation, as shown by DNA direct sequencing (TAC → CAC; Tyr → Asp; Fig 5 and Table 3).

DISCUSSION

The molecular characterization of cutaneous B- and T-cell lymphomas may have important implications in the classification and diagnosis of these diseases and may help to clarify the biologic origin of these neoplasias. With this aim in mind, we have performed a comprehensive molecular analysis of immune genes, oncogenes, and p53 tumor-suppressor gene in a large panel of CLs.

We detected a germline arrangement of the immune genes in 31 of the 52 CBCLs (≈60%) and in 1 of the 38 CTCLs (≈3%). Immunoglobulin genes have been extensively investigated in CLs of B and T lineage, and the absence of clonal rearrangements in these neoplasias, as well as in other noncutaneous B-cell NHLs, has been reported in 10% to 50% of the investigated cases.66-83 This wide range of variability might be due to different reasons, such as the possible diagnostic inadequacy of the biopsy specimen analyzed47 or the inclusion of different proportions of patients with early tumor lesions in whom the number of neoplastic cells could have been under the detection threshold of Southern blot. In our panel, diagnostic inadequacy of the biopsy specimen analyzed could account for these cases expressing surface Ig without detectable rearrangement of Ig genes. Other possible explanations have also been proposed; in particular, it has been suggested that lymphoid neoplasms with immune genes in germline configuration may represent the clonal expansion of very early cells frozen at a prearrangement stage of differentiation, as has been documented for prethymic nodal T-lymphoblastic lymphomas, or that immune gene deletion may have occurred during neoplastic progression.46,49 The former hypothesis may fit the ALCL case in our panel, which showed a germline arrangement of TCR β and γ genes and an immature phenotypic picture; the latter could be proposed for a fraction of CBCLs in our panel that are thought to represent mature B-cell neoplasms. In this regard, it is worth noting our finding that 4 CBCL cases showing IgH and light chain loci in germline configuration presented oncogene rearrangements (see below) that indicate the presence of a detectable clonal neoplastic population in the biopsy specimen analyzed. As far as we know, this is the first report in which CBCLs with Ig genes in germline configuration presented a near marker of clonality, supporting the hypothesis of the occurrence of Ig genes deletion in these 4 cases. Furthermore, the finding that tumors with Ig genes in germline configuration presented oncogene alterations indicates that any selection of cases on the basis of the rearrangement of immune genes may lead to underestimate the molecular lesions in these neoplasias.

Immunohistochemical and genotypical studies have suggested that primary CBCLs represent a distinct biologic entity among B-cell NHLs, characterized by a favorable clinical prognosis.29-31 In particular, the lack of CD10 reactivity,32,33 the absence of bcl-2 gene rearrangement,32-34 and the weak cytoplasmic expression or the absence of the bcl-2 protein35,36 (this study) represent features that differentiate primary CBCLs with a morphology suggesting a germinal center origin from the corresponding nodal NHLs.31,34,35,86 We provide here further evidence that structural alterations of the bcl-2 gene are involved in the pathogenesis of a limited proportion of CBCLs, confirming and extending the findings relating to this gene previously reported by us and others.32,34,35 In addition, our study indicates that the involvement of bcl-1 locus also represents a rare event in CBCLs; this finding, together with the constant absence of CD5 antigen expression, strongly argues against a possible origin of the neoplastic cells from the mantle zone. Interestingly, clonal rearrangement of Ig genes, the absence of CD5 and CD10 antigen expression and of bcl-1 and bcl-2 loci involvement, together with the expression of CD32 antigen, have been proposed as features characterizing MALT-derived B-cell lymphomas, for which an origin from monocytoid B cells of the marginal zone has been suggested.41,43,44 On the basis of these similarities, it has been proposed that a subset of CBCLs may originate from the marginal zone lymphocytes of the skin-associated lymphoid tissue (SALT),29,30 thus representing the cutaneous counterpart of MALT-derived lymphomas. Our data may support this hypothesis, at least in those CBCLs with rearranged Ig genes, and suggest that CBCLs could represent distinct clinicobiologic entities from the nodal lymphomas with which they might share common morphologic characteristics. In accordance with these observations, it is worth noting that rearrangements of the bcl-6 gene, a genetic lesion mainly associated with nodal diffuse large-cell lymphomas (DLCLs),77,92 have not been observed in the group of CBCL patients with similar histologic features. On the other hand, our analysis, which is the first study of bcl-6 gene involvement in CBCLs, detected a rearrangement affecting this gene in only 1 case that showed a lymphocytic histotype, suggesting that this genetic lesion represents a rare event in the pathogenesis of these neoplasias. However, the clinical implications of bcl-6 alterations in lymphoid neoplasias, and in particular their prognostic role, remain to be further investigated.92,93

Rearrangements of the tal-1 gene, a member of the bHLH family of transcription factors,80 were detected in 3 cases of CTCLs. In normal bone marrow, this gene is expressed in immature myeloid cells and in the erythroid, megakaryocytic, and mastocytic/basophilic lineages, but not in normal T lymphocytes.94,95 Interestingly, structural alterations of its locus have been almost exclusively associated with αβ lineage T-ALLs,92,96 and it has been proposed to be caused by illegitimate V(D)J recombination.97 Two distinct mechanisms account for tal-1 gene rearrangements: the chromosome translocation t(1:14)(p32;q11) involving the TCR-γδ gene complex and submicroscopic deletions that place the tal-1 gene under the control of SIL gene promoter.97-98 In both cases, altered tal-1 alleles are transcriptionally active,99 and an inappropriate expression of tal-1 protein has been proposed to be involved in the process of leukemogenesis.98 The presence of tal-1 gene deletions in CTCLs of our series, together with the unusual clinical aggressiveness of the cases.
bearing this alteration, extends the spectrum of \( tal-1 \) gene rearrangements to neoplasms other than \( T-ALL \) and suggests the pathogenetic role of inappropriate \( tal-1 \) gene expression in a significant proportion of CTCLs.

\( p53 \) inactivation by gene mutation is the most frequent genetic lesion in human cancers and has been frequently associated with tumor progression. Among lymphoid malignancies, \( p53 \) mutations have been reported to occur in advanced stages of multiple myeloma (MM) and in a minority of malignant NHLs, either highly malignant or transformed forms, or less aggressive tumors, such as B-CLLS and splenic lymphomas of marginal zone origin.

The frequency and type of \( p53 \) gene mutations in CTCLs have still not been extensively investigated. In the present study, \( p53 \) gene mutations were detected in only 1 case of primary CTCL (a tumoral stage MF), suggesting that \( p53 \) inactivation might play a very limited pathogenetic role in CTCLs. These findings could be explained by the peculiar clinical features of these neoplasias. Primary CBCLs are in fact characterized by a low grade of malignancy, indolent clinical course, possible spontaneous resolution of some lesions, and a generally good prognosis. Furthermore, the majority of CTCLs are characterized by a similar clinical behavior, including those forms expressing CD30 antigen, such as ALCLs and PTLs, which have highly malignant histologic features.

The \( NFkB2/lyt-10 \) gene, a member of the NF-xB transcription factor family, has recently been identified as a putative proto-oncogene involved in structural abnormalities of chromosome band 10q24, a recurrent cytogenetic aberration occurring in lymphoid malignancies. Our analysis showed the presence of molecular rearrangements in 2 of 52 (\( \approx 4\% \)) CBCL and 2 of 38 (\( \approx 5\% \)) CTCL cases. These three cases have been previously described at the molecular level in an extensive analysis of \( NFkB2/lyt-10 \) gene involvement in lymphoid neoplasias. The present study extends and modifies previously reported observations concerning the frequency of \( NFkB2/lyt-10 \) gene involvement in CTCLs by showing that, when assayed in a larger series of cases, it appears to be less frequent than previously reported. Nevertheless, together with data previously reported, these data suggest that \( NFkB2/lyt-10 \) gene rearrangements may represent a molecular event that preferentially targets CTCLs among the wide spectrum of lymphoid malignancies. Moreover, the specific clustering of breakpoints in the \( ankyrin \) domain, the expression of aberrant transcripts, and the modification of the DNA binding and functional properties of the in vivo rearranged protein further support the proposed pathogenetic role of \( NFkB2/lyt-10 \) gene in the tumorigenesis of these subsets of NHLs.

Finally, our analysis failed to detect any involvement of \( c-myc \) in CBCLs or of \( bcl-3 \) genes in either CBCLs or CTCLs. Our findings confirm and extend the previously reported absence of \( c-myc \) structural rearrangements in CBCLs, thus supporting the hypothesis that \( c-myc \) does not play a pathogenetic role in these tumors. Structural rearrangements of \( bcl-3 \) have not been previously investigated in CLs of B and T lineage, but our findings are in accordance with the results of a previous analysis of a large series of lymphoproliferative diseases, which indicated that \( bcl-3 \) gene rearrangements represent a rare event in these neoplasias.

In conclusion, our study, which was aimed at clarifying the molecular pathogenesis of CLs, indicates that molecular lesions frequently affecting nodal NHLs are involved in a limited fraction of CLs, suggesting that other molecular pathways may underlay the pathogenesis of these lymphoid neoplasias. These considerations may prompt the search of new and albeit unknown molecular markers characteristic of the majority of CLs. Furthermore, this study suggests the potential usefulness of a multiparametric approach based on clinical, histologic, immunophenotypic, and molecular analyses for the identification of biologically homogeneous subsets of CLs.

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