[CANCER RESEARCH 62, 4089–4094, July 15, 2002]

Alternative Translocation Breakpoint Cluster Region 5′ to *BCL*-6 in B-cell Non-Hodgkin's Lymphoma[1]

Marion P. Butler, Shinsuke Iida, Daniela Capello, Davide Rossi, Pulivarthi H. Rao, Palanisamy Nallasivam, Diane C. Louie, Seeta Chaganti, Thomas Au, Randy D. Gascoyne, Gianluca Gaidano, Raju S. K. Chaganti, and Riccardo Dalla-Favera[2]

Institute for Cancer Genetics, and the Departments of Pathology and Genetics & Development, Columbia University, New York, NY 10032 [M. P. B., S. I., R. D-F.]; Hematology Unit, Division of Internal Medicine, Department of Medical Sciences, Amedeo Avogadro University of Eastern Piedmont, 28100 Novara, Italy [D. C., D. R., G. G.]; Cell Biology and Genetics Programs, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [P. H. R., P. N., D. C. L., S. C., R. S. K. C.]; and the Department of Pathology, British Columbia Cancer Agency, Vancouver, V5Z 4E6 Canada [T. A., R. D. G.]

ABSTRACT

Chromosomal translocations involving band 3q27 with various different partner chromosomes represent a recurrent cytogenetic abnormality in B-cell non-Hodgkin's lymphoma. In a fraction of these translocations, the chromosomal breakpoint is located within the 5′ noncoding region of the *BCL*-6 proto-oncogene where the *BCL*-6 major breakpoint region (MBR) maps. As a result of the translocation, *BCL*-6 expression is deregulated by promoter substitution. However, between 30 and 50% of lymphomas with cytogenetically detectable translocations affecting band 3q27 retain a germ-line configuration at the *BCL*-6 locus. To identify possible additional breakpoint clusters within 3q27, we cloned a t(3;14)(q27;q32) lymphoma without MBR rearrangement and found a novel breakpoint site located between 245 and 285 kb 5′ to *BCL*-6. Breakpoints within this newly described region, which we called the alternative breakpoint region (ABR), were found to be recurrent in lymphomas carrying t(3q27) chromosomal translocations but devoid of *BCL*-6 MBR rearrangements. Comparative analysis of multiple lymphomas carrying rearrangements within the ABR showed that the breakpoints cluster within a 20-kb distance. Translocations involving the ABR may juxtapose *BCL*-6 to distantly acting, heterologous transcriptional regulatory elements which cause deregulation of the proto-oncogene. The identification of *BCL*-6 ABR provides new tools for the diagnosis of lymphomas carrying aberrations at 3q27 and deregulated *BCL*-6 genes.

INTRODUCTION

B-cell NHL[3] form a heterogeneous group of neoplasms that arise as a result of the clonal expansion of a single cell arrested at a specific stage of B-cell differentiation (1). The uncovering of the genetic elements involved in lymphomagenesis has been aided by cytogenetic analysis, which has revealed that nonrandom chromosomal translocations are found in association with specific subtypes of NHL (1–2). The molecular cloning of the breakpoint junctions of these translocations has led to the identification of several proto-oncogenes that undergo transcriptional deregulation because of their juxtaposition to regulatory elements of genes constitutively expressed in mature B cells, most often represented by Ig genes (1). The B-cell NHL-associated oncogenes discovered to date include *BCL*-1 at 11q13 involved in ~95% of mantle cell lymphomas, *BCL*-2 at 18q21 involved in 70–90% of FLs (1), c-*MYC* at 8q24, which is found to be rearranged in 100% of Burkitt's lymphoma (1), *PAX*-5 at 9p13 involved in 50% of lymphoplasmacytoid lymphomas (1), and *BCL*-6 at 3q27 rearranged in 30–40% of DLBCLs and in 4–15% of FL cases (3–5).

The *BCL*-6 gene encodes a nuclear phosphoprotein characterized by six COOH-terminal Kruppel-type zinc finger motifs, and an NH_2-terminal POZ motif, shared by several zinc finger molecules, including the Drosophila developmental regulators *Tramtrak* and *Broad-Complex*, as well as the human *KUP*, *ZID*, and *PLZF* proteins (3). BCL-6 has been shown to function as a potent transcriptional repressor of promoters linked to its DNA target sequence (6–8). The BCL-6 protein is expressed in mature B-cells within germinal centers but not in immature B-cell precursors or in differentiated plasma cells (9). Targeted disruption of the *BCL*-6 gene shows that *BCL*-6 is essential for germinal center formation, being involved in the control of Th2-type immune responses (10, 11).

Rearrangements involving the *BCL*-6 gene at 3q27 cluster mainly in a 4-kb genomic region, termed the MBR, which spans the first noncoding exon of the gene (3–5). Translocations affecting band 3q27 in NHL are not limited to the Ig loci but may involve numerous different partner chromosomes. The molecular characterization of several 3q27 chromosomal translocations has shown that the coding domain of the translocated *BCL*-6 gene becomes fused downstream to heterologous promoters. These include the Eμ and Iγ promoters of Ig genes in the case of t(3;14) as well as promoters of other genes, namely *TTF*, *BOB*-1, *H4*, in the case of t(3;4), t(3;11), and t(3;6), respectively (12–15). A general feature of promoters juxtaposed to translocated *BCL*-6 is their constitutive expression in B-cells, which leads to the inappropriate expression of BCL-6 (12–15).

Between 30 and 50% of the 3q27 chromosomal breakpoints found in lymphomas do not occur within the MBR of *BCL*-6, suggesting the presence of alternative breakpoints at 3q27 (3). To address this question, we cloned a t(3;14)(q27;q32) occurring in a FL case shown to be negative for rearrangement at the MBR of *BCL*-6. We detected a novel breakpoint cluster located 245–285 kb upstream of the first exon of the *BCL*-6 gene and termed ABR. This breakpoint site was shown to be recurrent in several lymphomas with 3q27 chromosomal translocations that lacked *BCL*-6 MBR rearrangement.

MATERIALS AND METHODS

Tumor Biopsies and Cell Lines. Lymph node biopsies from NHL cases were obtained from the Memorial Sloan-Kettering Hospital. Tumors were classified histologically according to the Revised European-American Lymphoma Classification (16). The t(3;14)(q27;q32)-positive NHL case 1952, which served for *BCL*-6 ABR cloning, was diagnosed as having a FL demonstrating an add (8)(q24) but no karyotypic evidence of a t(14;18)(q32;q21)

Received 2/1/02; accepted 5/17/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by the National Institute of Health Grant CA-37295 (to R. D-F.); Istituto Superiore di Sanità, III Programma Nazionale di Ricerca sull'AIDS 1999 - Progetto Patologia, Clinica e Terapia dell'AIDS, Rome, Italy (to G. G.); and Fondazione "Piera Pietro e Giovanni Ferrero," Alba, Italy (to G. G.). M. P. B. was supported in part by the Mater College, Dublin, Ireland, and the Vocational Education Committee Co., Donegal, Ireland. M. P. B. was registered in the PhD program of medicine in University College Dublin, Ireland. D. C. is supported by a fellowship from Federazione Italiana Ricerca Cancro, Milan, Italy.

[2] To whom requests for reprints should be addressed, at Institute for Cancer Genetics, Columbia University, Russ Berrie Science Pavilion, 1150 Saint Nicholas Avenue, Room 303B, New York, NY 10032. Phone: (212) 851-5273; Fax (212) 851-5256; E-mail: rd10@columbia.edu.

[3] The abbreviations used are: NHL, non-Hodgkin's lymphoma; Ig, immunoglobulin; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MBR, major breakpoint region; ABR, alternative breakpoint region; FISH, fluorescent *in situ* hybridization; PFGE, pulsed-field gel electrophoresis.

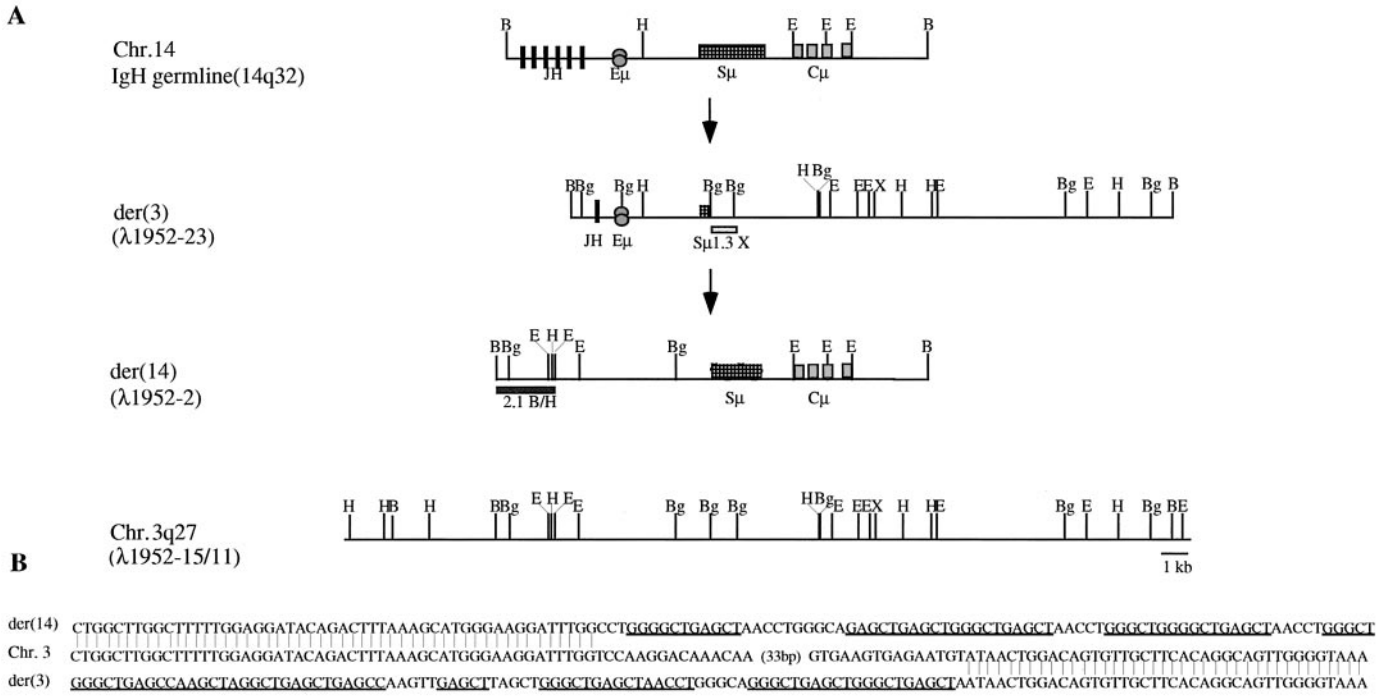

Fig. 1. Molecular characterization of the t(3;14) lymphoma case 1952. In *A*, the figure shows mapping of the recombinant phage clones λ1952-23 and λ1952-2, representing der(3) and der(14) respectively, in addition to the normal germ-line phages λ1952-15/11 at the *BCL*-6 ABR. Structural elements include the Ig enhancer element (Eμ), as well as the switch region (Sμ) and coding segments (Cμ and JH). *Arrows* indicate the position of the breakpoint in case 1952. Probes 2.1 B/H and 1.3 X are represented as thick horizontal lines and were used to confirm chromosome 3 derivation by hybridization to somatic cell hybrid DNA filters. In *B*, the figure shows the nucleotide sequence analysis of the breakpoint junctions of case 1952 and alignment to the corresponding germ-line regions. Sequence identities are indicated by the vertical lines. The sequences absent in der(3) and der(14), but present in germ-line chromosome 3, are the result of a deletion during translocation. Sμ motifs are *underlined*. Restriction enzyme abbreviations are: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; and Bg, *Bgl*II.

translocation. Immunohistochemical analysis demonstrated that this tumor expressed surface IgM.

Southern and Northern Blot Analysis. High molecular weight DNA was extracted by the SDS/proteinase K method followed by "salting out" and ethanol precipitation. Southern blot analysis was performed following standard procedures. The following probes were used for Southern blot analysis of Ig gene rearrangements: J_H probe, represented by a 6.6-kb *Bam*HI-*Hin*dIII fragment from the Ig_H locus (17); and Cμ probe, represented by a 1.3-kb *Eco*RI fragment from the Ig_H locus (17). Some of the *BCL*-6 ABR probes used in this study have been used in a previous study (18). For Northern blot analysis, RNA was extracted from cell lines and patient samples using the guanidinium thiocyanate/cesium chloride method, electrophoresed on a 1.0% agarose gel containing 2.2 mM formaldehyde and transferred to a nylon membrane (Hybond N; Stratagene).

Cloning of Chromosomal Breakpoints. High molecular weight DNA from NHL case 1952 was digested with *Bam*HI and size fractionated by running on a 0.7% low-melting agarose gel. Fragments ranging from 12 to 23 kb were gel-purified and ligated into the λDash-II vector (Stratagene, La Jolla, CA) and subsequently *in vitro* packaged using Gigapack III Gold packaging extract (Stratagene, La Jolla, CA). Recombinant phage clones (1×10^6) were screened by plaque hybridization using the J_H and Cμ Ig probes. Isolation of germ-line 3q27 sequences was achieved by screening a human placenta library (Stratagene) with probe 2.1 B/H derived from phage λ1952–2 {corresponding to der(14)} (Fig. 1).

Isolation of P1/PAC and YAC Clones and Construction of a 3q27 Contig. To establish a clone contig encompassing the *BCL*-6 gene, the Sac 4.0 probe (intron 1 of *BCL*-6) was used to screen a gridded P1 and PAC library (Genome Systems, St. Louis, MO). P1 and PAC clone ends were isolated by a vectorette-bubble PCR method and then used for genomic "walking" by additional screening of P1 and PAC libraries (19). The single YAC clone 19GA10 described in the contig, isolated from the Zeneca (formerly ICI) human YAC library, was obtained from the United Kingdom Human Genome Mapping Project Resource Centre, Hinxton, United Kingdom. Confirmation that the isolated P1 and PAC clones were from chromosome 3 was achieved through the hybridization of PCR-derived end clone fragments to somatic cell hybrid filters (panel 2, National Institute of General Medical Sciences Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ), as well as FISH analysis to metaphase spreads of mitogen-stimulated normal human lymphocytes. Insert sizing of P1 and PAC clones was achieved through their digestion with *Not*I/*Sal*I and *Not*I restriction digests, respectively. For YAC clones, yeast cells were embedded in agarose plugs by a standard method and analyzed by PFGE on the CHEF-DR II (Bio-Rad, Richmond, CA) after restriction digestion.

FISH. Briefly, probes were labeled by nick translation with biotin-16-dUTP or digoxigenin-11-dUTP and detected by indirect immunofluorescence using fluorescein isothiocyanate-conjugated avidin and rhodamine-conjugated anti-digoxigenin, respectively. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole. Images were captured with a cooled charge-coupled device camera (Photometrics, Tucson AZ) attached to a Nikon Microphot-SA microscope and processed using Smart capture imaging system (Vysis, Downers Grove, IL).

DNA Sequencing. DNA sequencing was performed by the dideoxy chain termination method using the ABI 373A automated sequencing system (Applied Biosystems, Foster City, CA).

RESULTS

Cloning of t(3;14) from a FL. Cloning of t(3;14)(q27;q32) was performed on case 1952, represented by an IgM-producing FL. Southern blot analysis of Ig genes using *Bam*HI and *Hin*dIII digestions and probes for J, Cμ, Cγ, and Cα sequences showed the presence of three rearranged J fragments, two of which did not comigrate with any constant gene region probe (data not shown) as observed previously in several cases of chromosomal translocations involving the Ig_H locus (20). We then proceeded to clone the rearranged *Bam*HI fragments containing J_H and Cμ sequences from a recombinant phage library constructed from *Bam*HI-digested DNA from case 1952, which was

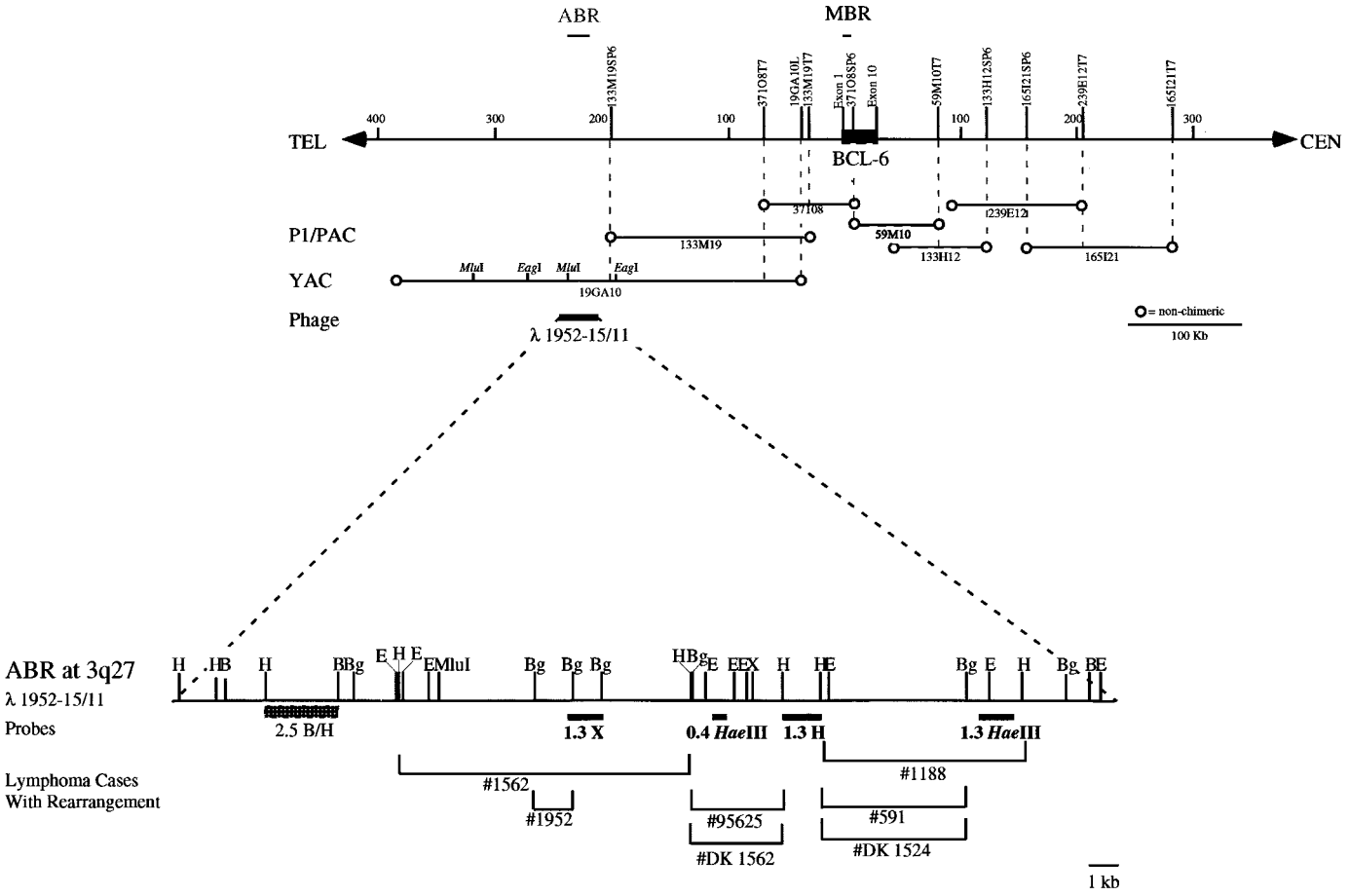

Fig. 2. Physical map of the 3q27 genomic region encompassing the *BCL*-6 gene. The *top panel* shows a contig of P1, PAC, and YAC clones which covers a genomic region of ~650 kb. The insert size of these reagents was determined by PFGE. The *open circles* represent nonchimeric clones as determined by hybridization of the PCR-derived end fragments to somatic cell hybrid DNA panels. The extent of overlap between adjacent P1 and PAC clones was achieved through restriction fingerprint analysis. The *BCL*-6 gene is represented as the *filled box*, exons 1 and 10 are indicated. The ABR is represented as a *thick horizontal line* (λ1952-15/11), the detailed schematic representation of which is shown in the *bottom panel*. In the *bottom panel*, the *black horizontal bars* represent the probes used to assess for frequency of rearrangement at the ABR in the tumor cases. The fragment 2.5 B/H (▨) was used as a probe in Southern blot hybridization analysis to map the location of the ABR. The lymphoma cases positive for rearrangement at the ABR are indicated on the map, and rearrangements are defined within *Hin*dIII restriction fragments. Restriction enzyme abbreviations are: B, *Bam*HI; H, *Hin*dIII; Bg, *Bgl*II; and E, *Eco*RI.

screened by both J_H and Cμ probes. Restriction mapping and hybridization analysis revealed that the recombinant phage clones contained J_H and Cμ sequences juxtaposed to sequences unrelated to the Ig_H locus. To determine the chromosomal origin of these sequences, a 2.1-kb *Bam*HI-*Hin*dIII fragment from λ1952-2, as well as a 1.3-kb *Xba*I fragment from λ1952-23 (Fig. 1), were hybridized to DNA of somatic-cell hybrids representative of individual human chromosomes. Both probes identified human sequences only on somatic cell hybrids containing chromosome 3 (data not shown). Probe 2.1 B/H from λ1952-2 was then used to screen a genomic placenta library to clone the corresponding normal locus on chromosome 3, represented by phage clones λ1952-15 and λ1952-11 (Fig. 2).

Restriction mapping and partial sequence analysis of der(3) and der(14) indicated that the clones represented a reciprocal translocation (Fig. 1). The position of the breakpoints was mapped by sequencing of derivatives (3) and (14) junctions regions, which revealed that the recombination involved a deletion of 63 bp on chromosome 3 (Fig. 1). FISH analysis of metaphase spreads prepared from case 1952 using the phage contig (γ1952-15/11) as a probe revealed signals on chromosomal derivatives (3) and (14) as well as normal chromosome 3, band q27, thus confirming the derivation of these phages from a t(3;14) (data not shown).

By sequencing analysis, the typical Ig switch pentameric repeat motifs were seen in the immediate vicinity of the cloned breakpoint. No apparent homology was detectable between chromosome 14 and chromosome 3 sequences near the breakpoint.

Relationship between the 3q27 Breakpoint of Case 1952 and the *BCL*-6 Gene. To define the position of the newly identified 3q27 breakpoint, a contig of P1, PAC, and YAC clones was constructed linking the newly cloned ABR to the *BCL*-6 locus (Fig. 2). A restriction map of the YAC clone 19GA10 is given in Fig. 2, showing *Mlu*I and *Eag*I restriction enzyme sites identified by double enzyme digestion and by hybridization analysis using YAC/PAC clone ends. By Southern blot hybridization analysis, probe 1.3 X from ABR (Fig. 2) hybridized only to YAC clone 19GA10 in the contig. It hybridized specifically to a 220-kb *Mlu*I restriction fragment of YAC clone 19GA10. An adjacent ABR probe, termed 2.5 B/H, hybridized to a different *Mlu*I restriction fragment of 85 kb (see Fig. 2). This indicates that the ABR is located 220 kb from the left YAC arm terminus, 19GA10L. By Southern blot hybridization analysis, probe 19GA10L hybridizes to both P1371O8 and PAC133M19. The 5′ marker of P1371O8 (T7 end) is positioned ~65 kb 5′ to exon 1 of BCL-6 by a combination of PFGE and hybridization analysis (Fig. 2). Marker 133M19T7 maps 25 kb 5′ to *BCL*-6 as determined by restriction fingerprint analysis and hybridization analysis (data not shown). Overall, these results indicate that ABR is positioned at a distance of 245–285 kb 5′ to *BCL*-6.

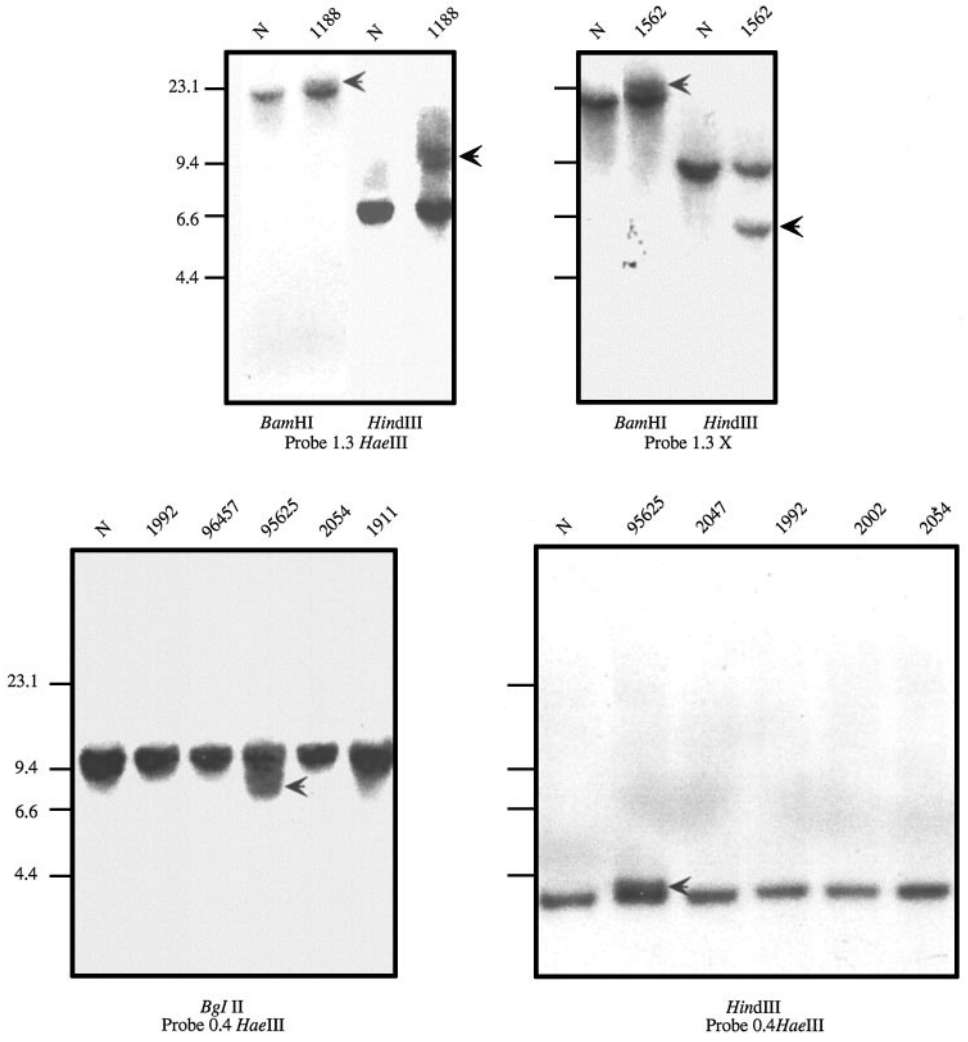

Fig. 3. Southern blots showing rearrangement at the *BCL*-6 ABR in three representative lymphoma cases. Rearrangements are depicted in lymphoma cases 1188, 1562, and 95-625, confirmed by two DNA digests.

Recurrency of the BCL-6 ABR in Lymphomas. To assess the frequency of ABR, we collected a series of lymphomas ($n = 12$) with cytogenetic evidence of t(3q27) translocations but absence of rearrangement involving the MBR of *BCL*-6. The histological and partial cytogenetic profiles of these NHL cases are presented in Table 1. Southern blots of *Hin*dIII-, *Bgl*II-, and *Bam*HI-digested tumor DNAs were prepared and hybridized sequentially with probes derived from phages λ1952-15/11, exploring a genomic distance of ~40 kb surrounding the breakpoint of case 1952. All rearrangements reported were confirmed by analysis with a second enzyme digest. Representative results of hybridization analyses with probes from the ABR on Southern blots of t(3q27) NHL cases are shown in Fig. 3. Using probes 1.3 X and 0.4 *Hae*III on *Hin*dIII, *Bgl*II, and *Bam*HI Southern blot digests of lymphoma cases (Fig. 3), rearrangements were seen in 42% (5 of 12) of NHL cases that had cytogenetic evidence of t(3q27) chromosomal translocations and that showed germ-line configuration of the MBR (Table 1).

Table 1 *Rearrangement analysis at ABR in NHL cases with 3q27 chromosomal translocations*

Case	Histology	3q27 aberration	ABR	MBR
1952	FL	t(3;14)(q27;q32)	R	G
1562	DLBCL	t(2;3)(q21;q27) t(3;8)(q27;q13)	R	G
591	DLBCL	t(3;22)(q27;q11)	R	G
95-625	DLBCL	t(3;14)(q27;q32)	R	G
1188	DLBCL	add(3q27)	R	G
498	DLBCL	t(3;9)(q27;p13)	G	G
1187	FL	t(2;3)(q21;q27)	G	G
770	DLBCL	add(3q27)	G	G
1879	FL	t(3;14)(q27;q32)	G	G
95-517	DLBCL	t(2;3)(q12;q27)	G	G
95-89	DLBCL	t(3;6;14)(q27;p35;q32)	G	G
91-153	DLBCL	t(3;14)(q27;q32)	G	G

Subsequently, we investigated a cytogenetically uncharacterized series of FL ($n = 20$) and DLBCL ($n = 84$) that were known to be devoid of *BCL*-6 MBR rearrangements. Rearrangements of the *BCL*-6 ABR were detected in 10% (2 of 20) FL and 2.4% (2 of 84) DLBCL, including one case arising in an immunocompetent host and one case associated with AIDS. Mapping studies showed that all ABR rearrangements of *BCL*-6 clustered within a 20-kb region.

BCL-6 Expression Levels in NHL Cases with ABR Rearrangement. To analyze the effect of ABR rearrangement on *BCL*-6 gene expression, we examined the levels of *BCL*-6 expression in lymphoma cases 1952 (FL) and 1188 (DLBCL) (both rearranged at ABR), as compared with two control FLs (cases 2065 and 1992) showing no rearrangement at the ABR or MBR of *BCL*-6 (Fig. 4). *BCL*-6 RNA levels were similar, independent of the *BCL*-6 genomic status. In addition, *BCL*-6 expression levels of case 1952 were also comparable with those of DLBCL cell line LY18 with germ-line *BCL*-6, as well as of immunoblastic lymphoma LY8 carrying a MBR rearrangement. Overall, these results indicate that

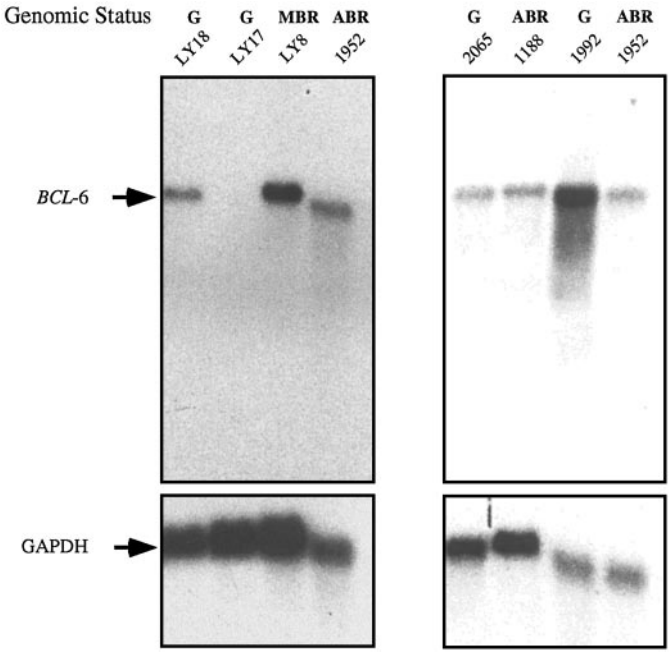

Fig. 4. Northern blot analysis of the t(3;14) positive lymphoma case 1952 hybridized with the *BCL*-6 probe. Other lymphoma cases are also included for comparison. A 10-μg aliquot of total RNA was loaded onto each lane, blotted, and hybridized with the full-length *BCL*-6 cDNA probe together with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe to control for the amount of RNA loading, as well as RNA degradation for the tumor RNAs. LY18 and LY17 are DLBCL cell lines; LY8 is an immunoblastic lymphoma cell line; primary cases 2065, 1992, and 1952 are FL; case 1188 is a DLBCL. The genomic status of each sample is indicated as ABR; MBR; G (for germ-line configuration at the ABR and MBR of *BCL*-6).

cases with ABR rearrangement express *BCL*-6 at levels comparable with phenotypically similar cases with MBR rearrangement or lacking 3q27 abnormalities.

DISCUSSION

B-cell NHL are typically characterized by recurrent chromosomal translocations, which deregulate proto-oncogenes found at or in close proximity to the chromosomal breakpoint junction areas (1). In some translocations, exemplified by the *BCL*-1 and *BCL*-2 translocations, the proto-oncogene may be involved by multiple, distinct breakpoint clusters that are recurrent in different patients (21, 22). In the case of 3q27 translocations involving the *BCL*-6 proto-oncogene, only one breakpoint cluster has been identified to date (3). This breakpoint cluster, termed *BCL*-6 MBR, is detectable at the molecular level in ~50–60% of lymphoma cases carrrying the 3q27 translocation (3–5). The occurrence of lymphomas harboring 3q27 breaks but devoid of *BCL*-6 MBR rearrangements point to the putative existence of other *BCL*-6 breakpoint clusters. Here we report the identification of a novel ABR, which, together with the *BCL*-6 MBR, accounts for ~70% of the total number of 3q27 chromosomal breakpoints detectable in NHL cases.

The *BCL*-6 ABR is located at a distance of ~280 kb telomeric to *BCL*-6, raising the question of how the translocation induces *BCL*-6 deregulation. On the basis of the model of several other lymphoma translocations, the ABR breaks may exert several possible modes of *BCL*-6 deregulation. These include substitution of enhancer regions, a mechanism which has been well demonstrated for many B-cell-associated oncogenes, including *BCL*-1, *BCL*-2, c-*MYC*, *PAX*-5, *BCL*-8, and *BCL*-9 (1). Enhancer substitution results from the insertion of various Ig_H transcriptional control elements, such as the potent J_H-C_H intron enhancer Eμ, the 3′αE, or the 3′α-hs4, at the junction region of the translocation breakpoint (23). An alternative mode of *BCL*-6 deregulation by ABR translocations consists in the removal of *cis*-acting regulatory domains in a fashion similar to that suggested for some rearrangements involving the c-*MYC* locus (24). In the case of NHL cases rearranged at the ABR but not involving the Ig genes, it is conceivable that *BCL*-6 is deregulated by enhancer substitution of genes other than Ig genes. Finally, it cannot be formally excluded that ABR breaks affect genes mapping to 3q27 but unrelated to *BCL*-6. However, the genomic region surrounding ABR analyzed to date failed to reveal any functionally proven gene.

Our survey of *BCL*-6 ABR breakpoints in B-cell lymphoma defines that the frequency of ABR rearrangements is substantially lower than that of *BCL*-6 MBR. In fact, MBR rearrangements seem to be involved in ~75% of DLBCL cases with cytogenetically detectable 327 breakpoints (3q27+) (3), whereas ABR rearrangements account for only a fraction (4 of 7, >50% in this study) of the remaining MBR-negative 3q27+ cases. Thus, ABR rearrangements are expected to account for ~12% of all 3q27 rearrangements, corresponding to an expected frequency of 4% in the overall DLBCL populations and consistent with the actual frequency observed in this initial study (2 of 84). Conversely, it is curious that the frequency of ABR rearrangements in FL appears to be similar, or possibly higher, than that of MBR rearrangements. Future studies on large panels of cases are needed to define whether *BCL*-6 ABR rearrangements preferentially associate with FL.

From a diagnostic standpoint, the results of this study point to the need of long range DNA analysis for the correct identification of all *BCL*-6 breakpoints occurring in lymphomas. In this respect, FISH provides a powerful tool to detect both MBR and ABR *BCL*-6 breakpoints, thereby surpassing the capabilities of conventional Southern blot hybridization analysis. In particular, highly predictive analysis of *BCL*-6 rearrangements can be performed by FISH using as a probe a 650-kb clone contig designed to detect breakpoints occurring both at the MBR and ABR, as well as those which scatter around the *BCL*-6 locus. Because a fraction of 3q27 breaks cannot be currently explained by *BCL*-6 breaks at MBR or ABR, FISH studies may also lead to the discovery of additional *BCL*-6 breakpoint clusters that, together with MBR and ABR, will recapitulate the molecular pathology of 3q27 breaks in B-cell lymphoma.

REFERENCES

1. Gaidano, G., and Dalla-Favera, R. Pathobiology of non-Hodgkin's lymphomas. *In:* R. Hoffman, E. J. Benz, Jr., S. J. Shattil, B. Furie, H. J. Cohen, L. E. Silberstein, and P. McGlave (eds.), Hematology. Basic Principles and Practice, Ed. 3, pp. 1213–1229. New York: Churchill Livingstone, 2000.
2. Koduru, P. R., Filippa, D. A., Richardson, M. E., Jhanwar, S. C., Chaganti, S. R., Koziner, B., Clarkson, B. D., Lieberman, P. H., and Chaganti, R. S. K. Cytogenetic and histologic correlations in malignant lymphoma. Blood, *69:* 97–102, 1987.
3. Ye, B. H., Lista, F., Lo Coco, F., Knowles, D. M., Offit, K., Chaganti, R. S. K., and Dalla-Favera, R. Alterations of a zinc finger-encoding gene, *BCL*-6, in diffuse large-cell lymphoma. Science (Wash. DC), *262:* 747–750, 1993.
4. Lo Coco, F., Ye, B. H., Lista, F., Corradini, P., Offit, K., Knowles, D. M., Chaganti, R. S. K., and Dalla-Favera, R. Rearrangements of the *BCL*6 gene in diffuse large cell non-Hodgkin's lymphoma. Blood, *83:* 1757–1759, 1994.
5. Bastard, C., Deweindt, C., Kerckaert, J. P., Lenormand, B., Rossi, A., Pezzella, F., Fruchart, C., Duval, C., Monconduit, M., and Tilly, H. LAZ3 rearrangements in non-Hodgkin's lymphoma: correlation with histology, immunophenotype, karyotype, and clinical outcome in 217 patients. Blood, *83:* 2423–2427, 1994.
6. Deweindt, C., Albagli, O., Bernardin, F., Dhordain, P., Quief, S., Lantoine, D., Kerckaert, J. P., and Leprince, D. The LAZ3/BCL6 oncogene encodes a sequence-specific transcriptional inhibitor: a novel function for the BTB/POZ domain as an autonomousrepressing domain. Cell Growth Differ., *6:* 1495–1503, 1995.
7. Chang, C. C., Ye, B. H., Chaganti, R. S. K., and Dalla-Favera, R. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. Proc. Natl. Acad. Sci. USA, *93:* 6947–6952, 1996.
8. Seyfert, V. L., Allman, D., He, Y., and Staudt, L. M. Transcriptional repression by the proto-oncogene BCL-6. Oncogene, *12:* 2331–2342, 1996.
9. Cattoretti, G., Chang, C. C., Cechova, K., Zhang, J., Ye, B. H., Falini, B., Louie, D. C., Offit, K., Chaganti, R. S. K., and Dalla-Favera, R. BCL-6 protein is expressed in germinal-center B cells. Blood, *86:* 45–53, 1995.

10. Ye, B. H., Cattoretti, G., Shen, Q., Zhang, J., Hawe, N., de Waard, R., Leung, C., Nouri-Shirazi, M., Orazi, A., Chaganti, R. S. K., Rothman, P., Stall, A. M., Pandolfi, P. P., and Dalla-Favera, R. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat. Genet., *16:* 161–170, 1997.
11. Dent, A. L., Shaffer, A. L., Yu, X., Allman, D., and Staudt, L. M. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science (Wash. DC), *276:* 589–592, 1997.
12. Ye, B. H., Chaganti, S., Chang, C. C., Niu, H., Corradini, P., Chaganti, R. S. K., and Dalla-Favera, R. Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. EMBO J., *14:* 6209–6217, 1995.
13. Dallery, E., Galiegue-Zouitina, S., Collyn-d'Hooghe, M., Quief, S., Denis, C., Hildebrand, M. P., Lantoine, D., Deweindt, C., Tilly, H., and Bastard, C. TTF, a gene encoding a novel small G protein, fuses to the lymphoma-associated LAZ3 gene by t(3;4) chromosomal translocation. Oncogene, *10:* 2171–2178, 1995.
14. Galieque-Zouitina, S., Quief, S., Hildebrand, M. P., Denis, C., Lecocq, G., Collyn-d'Hooghe, M., Bastard, C., Yuille, M., Dyer, M. J., and Kerckaert, J. P. The B cell transcriptional coactivator *BOB1*/*OBF1* gene fuses to the *LAZ3*/*BCL6* gene by t(3;11)(q27;q23.1) chromosomal translocation in a B cell leukemia line (Karpas 231). Leukemia (Baltimore), *10:* 579–587, 1996.
15. Akasaka, T., Miura, I., Takahashi, N., Akasaka, H., Yonetani, N., Ohno, H., Fukuhara, S., and Okuma, M. A recurring translocation, t(3;6)(q27;p21), in non-Hodgkin's lymphoma results in replacement of the 5′ regulatory region of BCL6 with a novel H4 histone gene. Cancer Res., *57:* 7–12, 1997.
16. Harris, N. L., Jaffe, E. S., Stein, H., Banks, P. M., Chan, J. K., Cleary, M. L., Delsol, G., De Wolf-Peeters, C., Falini, B., Gatter, K. C., Grogan, T. M., Isaacson, P. G., Knowles, D. M., Mason, D. Y., Müller-Hermelink, H-K., Pileri, S., Piris, M. A., Ralfkiaer, E., and Warnke, R. A. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood, *84:* 1361–1392, 1994.
17. Ravetch, J. V., Siebenlist, U., Korsmeyer, S., Waldmann, T., and Leder, P. Structure of the human immunoglobulin mu locus: characterization of embryonic and rearranged J and D genes. Cell, *27:* 583–591, 1981.
18. Chen, W., Butler, M., Rao, P. H., Chaganti, S. R., Louie, D. C., Dalla-Favera, R., and Chaganti, R. S. K. The t(2;3)(q21;q27) translocation in non-Hodgkin's lymphoma displays BCL6 mutations in the 5′ regulatory region and chromosomal breakpoints distant from the gene. Oncogene, *17:* 1717–1722, 1998.
19. Riley, J., Butler, R., Ogilvie, D., Finniear, R., Jenner, D., Powell, S., Anand, R., Smith, J. C., and Markham, A. F. A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acid. Res., *18:* 2887–2890, 1990.
20. Neri, A., Barriga, F., Knowles, D. M., Magrath, I. T., and Dalla-Favera, R. Different regions of the immunoglobulin heavy-chain locus are involved in chromosomal translocations in distinct pathogenetic forms of Burkitt lymphoma. Proc. Natl. Acad. Sci. USA, *85:* 2748–2752, 1988.
21. Cleary, M. L., Galili, N., and Sklar, J. Detection of a second t(14;18) breakpoint cluster region in human follicular lymphomas. J. Exp. Med., *164:* 315–320, 1986.
22. Meeker, T. C., Grimaldi, J. C., O'Rourke, R., Louie, E., Juliusson, G., and Einhorn, S. An additional breakpoint region in the BCL-1 locus associated with the t(11;14)(q13;q32) translocation of B-lymphocytic malignancy. Blood, *74:* 1801–1806, 1989.
23. Michaelson, J. S., Giannini, S. L., and Birshtein, B. K. Identification of 3′ a-hs4, a novel Ig heavy chain enhancer element regulated at multiple stages of B cell differentiation. Nucleic Acid. Res., *23:* 975–981, 1995.
24. Mautner, J., Behrends, U., Hortnagel, K., Brielmeier, M., Hammerschmidt, W., Strobl, L., Bornkamm, G. W., and Polack, A. c-myc expression is activated by the immunoglobulin κ-enhancers from a distance of at least 30 kb but not by elements located within 50 kb of the unaltered c-myc locus *in vivo*. Oncogene, *12:* 1299–1307, 1996.

Alternative Translocation Breakpoint Cluster Region 5′ to *BCL*-6 in B-cell Non-Hodgkin's Lymphoma

Marion P. Butler, Shinsuke Iida, Daniela Capello, et al.

Cancer Res 2002;62:4089-4094.