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Rapid Communication

The bcl-2 Gene in Primary B Cell Lymphoma of Mucosa-Associated Lymphoid Tissue (MALT)

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The bcl-2 gene rearrangement representing t(14:18) chromosomal translocation is the most frequent karyotypic abnormality in non-Hodgkin's lymphomas of follicle center-cell lineage. By using three bcl-2 DNA probes, 21 cases of non-Hodgkin's B cell lymphoma arising from gastrointestinal mucosa and eight cases of follicular lymphomas were examined. No rearrangement of the gene could be detected in the group of gastrointestinal lymphomas, although it was identified in 75% of the follicular lymphomas. The findings suggest that these two groups of lymphomas are not a family at genetic level and support the earlier suggestion that B cell lymphomas arising from gastrointestinal mucosa-associated lymphoid tissue are not of follicle center-cell lineage. (Am J Pathol 1989, 135: 7–11)

Analysis of the clinicopathologic features of primary B cell lymphomas arising in mucosal sites (principally the gastrointestinal tract) led to the proposal that they are a distinctive group of tumors sharing a common lineage that is not reflected in current classifications of non-Hodgkin's lymphomas.[1,2] This view differs with that expressed in numerous reports of large series of gastrointestinal lymphomas in which the tumors tend to be classified heterogeneously, usually encompassing the full range of the various classifications.[3–10] Follicle center-cell lymphomas are featured in these reports and Isaacson and Wright,[11] who first coined the term "malignant lymphoma of mucosa-associated lymphoid tissue (MALT)," and later Isaacson et al[12] concluded that these tumors, despite their unusual clinicopathologic features, were of follicle center-cell origin. This conclusion was based on the cytology of the diffuse component of the lymphomas that resembled that of cleaved follicle center-cells (centrocytes), leading to the term centrocyte-like (CCL) cell, and on the invariable presence of numerous follicles some of which could be judged to be neoplastic on either morphologic or immunohistochemical grounds. Later, in a critical immunohistochemical study of a series of primary gastric lymphomas Myhre and Isaacson[13] sought to show that the follicles in these tumors were uniformly reactive but were frequently colonized by the CCL cells, leading to an appearance that at its extremes could mimic true follicular lymphoma. These authors concluded that MALT lymphomas were not of follicle center-cell lineage and raised the possibility that they were of marginal zone B cell lineage.

Recent cytogenetic studies of non-Hodgkin's lymphoma have revealed a close association between certain histologic patterns and nonrandom chromosomal abnormalities.[14–18] Notable among these is the association between follicular low-grade non-Hodgkin's lymphoma and translocation involving chromosomes 14 and 18, t(14:18). This translocation results in the juxtaposition of the candidate proto-oncogene bcl-2 (B cell leukemia lymphoma) located at 18q21 with the immunoglobulin heavy chain (Jh) locus at 14q32.[19–21] Using chromosome-18 DNA probes flanking or within the bcl-2 gene, rearrangements of the gene representing t(14:18) translocations were detected in approximately 90% of follicular B cell lymphomas.[22] The gene rearrangements have also been detected in up to 28% of diffuse large B cell lymphomas, with the highest rate of detection in those tumors arising in a setting with a previous follicular histology.[22,23]

In this study, therefore, we sought the presence of the bcl-2 gene rearrangements in a series of 21 cases of primary B cell gastrointestinal lymphoma, all of which

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Table 1. *DNA Probes*

Probe	Name	Reference
Heavy chain; joining region (Jh)	C76R51A	29
bcl-2;		
Fragment adjacent to major break point	pFL1	20, 30
Major break point	pFL3	20, 30
Minor break point	pFL2	31

showed the histologic and immunohistochemical features of B cell lymphoma arising in MALT, comparing the results with a group of nodal follicular lymphomas and a single intestinal follicular lymphoma investigated in parallel. The presence of the gene rearrangements in a significant number of cases would provide strong evidence for a follicle center-cell lineage for these tumors.

Materials and Methods

Tissues were obtained from the frozen tissue bank of the Department of Histopathology, University College and Middlesex School of Medicine. The material included 17 cases of primary gastric lymphoma (12 low grade and five high grade), one case of low grade primary small intestinal lymphoma, and three cases of low grade Mediterranean lymphoma or immunoproliferative small intestinal disease (IPSID). All cases had been fully characterized morphologically and, with the exception of the IPSID cases, had been shown to exhibit light chain restriction.[12,13] Histologic details of all 12 cases of low grade gastric lymphoma were published previously[12,13] and, as previously described, follicles were a distinct component of these tumors, in one case imparting a follicular appearance to the tumor as a whole. Follicles were abundant in the single case of low grade small intestinal lymphoma and a minor component of two cases of IPSID. The third case of IPSID exhibited a striking follicular pattern as has been described in the so-called follicular variant of this disease.[24,25] The five cases of high grade gastric lymphoma were all diffuse. Eight cases of clear cut follicular lymphomas were included as controls, seven of which were from peripheral lymph nodes and one that arose as a primary tumor in the small intestine. This latter case demonstrated the classical morphologic and immunohistochemical features of follicular lymphoma (including CD10 positivity) without a diffuse component; lymphoepithelial lesions were not seen. Other controls included normal placenta, tonsil, and peripheral blood lymphocytes from the IPSID cases and normal tissue adjacent to one case of gastric lymphoma.

Standard procedures[26] were used to extract DNA from snap frozen tumor biopsy specimens. Purified DNA was digested separately with three restriction enzymes (EcoR1, HindIII, BamHI or BglII). DNA fragments were fractioned according to size in 0.8% agarose gels and were transferred to nylon membranes (Gene-Screen Plus, DuPont, HEN, Boston, MA) by Southern blotting.[27] The DNA probes to the bcl-2 and the immunoglobulin genes (Table 1) were radiolabeled with ^{32}P-dCTP by the random hexamer method[28] and hybridized to the membranes using the conditions recommended by the manufacturer. The membranes were washed at an appropriate stringency and exposed to prefogged x-ray films at −70 C for from 2 to 4 days.

Results

The results of Southern blot of the lymphomas tested with Jh and bcl-2 probes are summarized in Table 2. One or more rearranged Jh gene segments, together with a germline, were identified in the DNA from all the cases of lymphoma digested with three restriction enzymes (Figure 1A). No rearranged fragments could be detected in any of the cases of primary gastrointestinal lymphoma with any of the three bcl-2 probes (Figure 1B). Six of eight cases of follicular lymphoma, including the case arising in the small intestine, showed rearranged bcl-2 gene segments with each of the three restriction enzymes. Three of them were detected with pFL1/pFL3 probes (to the bcl-2 major breakpoint cluster region). The rearrangements in the other three cases were detected with pFL2 to the bcl-2 minor breakpoint cluster region (Figure 2). None of the cases showed bcl-2 rearrangement that was detectable by both pFL1/pFL3 and pFL2. In five of these six cases, it could be demon-

Table 2. *Jh and bcl-2 Gene Rearrangements in Gastrointestinal and Follicular Lymphomas*

Lymphoma type	Number	Jh (%)	pFL1/pFL3 (%)	pFL2 (%)
Follicular lymphoma*	8	8 (100)	3 (37.5)	3 (37.5)
Lymphoma of MALT				
Gastric				
High grade	5	5 (100)	0 (0)	0 (0)
Low grade	12	12 (100)	0 (0)	0 (0)
Small intestinal	1	1 (100)	0 (0)	0 (0)
IPSID	3	3 (100)	0 (0)	0 (0)
Total	21	21 (100)	0 (0)	0 (0)

* Seven from peripheral lymph nodes and one from the small intestine.

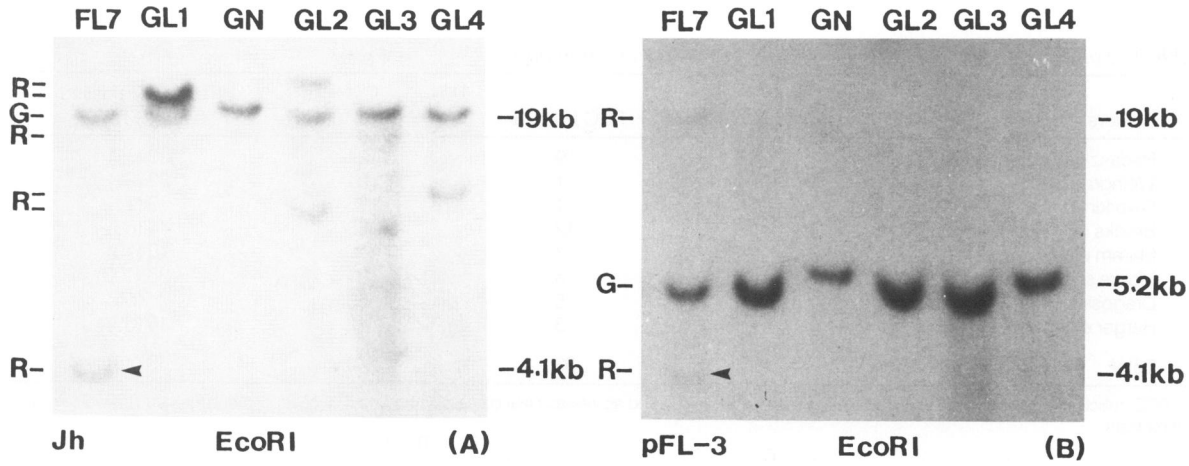

Figure 1. A: *Southern blot of genomic DNA from gastric lymphomas (GL1-4) and a follicular lymphoma (FL7) hybridized with probe Jh to the immunoglobulin heavy-chain gene-joining region.* B: *The same blot rehybridized with probe pFL3 to bcl-2 major breakpoint. GN, control DNA from uninvolved normal tissue adjacent to a gastric lymphoma (GL1). Rearranged fragments are indicated by R, germline by G. Arrowheads in A and B indicate comigrating, rearranged bands representive of t(14:18) DNA fragments.*

strated that the rearranged bcl-2 fragment comigrated with a rearranged immunoglobulin heavy-chain-joining region fragment, indicating t(14:18) translocations. Only germline could be identified with all the Jh and bcl-2 probes in control DNA from the placenta, tonsil, and peripheral blood lymphocytes from the IPSID cases and with the normal tissue adjacent to the gastric lymphoma.

Discussion

The bcl-2 gene rearrangements representing t(14:18) chromosomal translocation have been investigated recently in a wide range of heamatolymphoid malignancies.[19-23,31-33] It was reported that the gene rearrangements commonly occur in all non-Hodgkin's lymphomas of follicle center-cell lineage, especially in those with a follicular morphology.[22,23,32] In neoplasms not of follicle center-cell lineage, rearrangements of the gene are rarely detected.[22,23] All these data suggest that bcl-2 gene rearrangements are useful genetic markers for defining the histogenesis and improving the subclassification of lymphoid neoplasms.

In the present study, 75% of our follicular lymphoma cases showed bcl-2 gene rearrangements. The results are in accord with previously reported data, except that we found a higher incidence of rearrangements detected by pFL2, a probe to the bcl-2 minor breakpoint cluster region. This was possibly due to the random distribution in our relatively small number of follicular lymphoma cases or may reflect some geographic difference in our cases compared with those reported in the United States. Further studies of a large series of follicular lymphomas are needed to resolve this issue.

Figure 2. *Analysis of DNA from follicular lymphomas (FL1-6) with probe pFL2 to bcl-2 minor breakpoint. FL6 is a DNA from follicular lymphoma of the small intestine. DNA from normal tonsil tissue (T) was used as a control.*

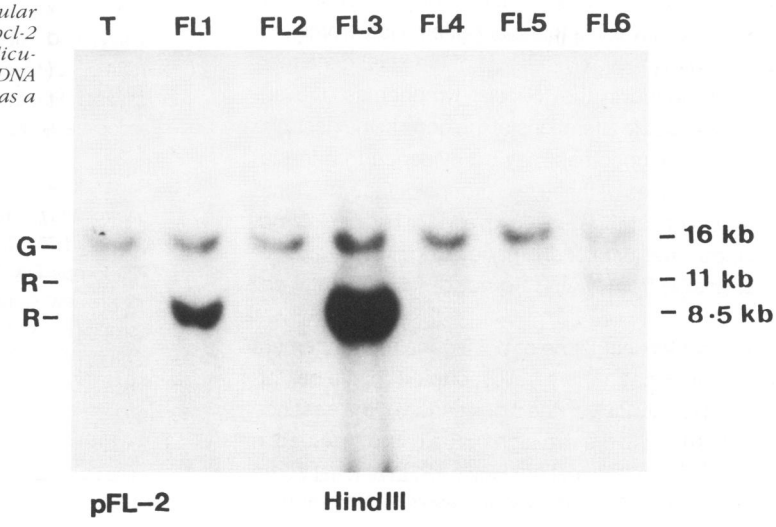

Table 3. *Follicle Center-Cell Lineage in Gastrointestinal Lymphoma*

Authors	Number	FCC follicular	FCC diffuse	Total FCC derived (%)
Radaszkiewicz and Dragosics[3]	60	9	21	30 (50)
Weingrad et al[4]	104	1	21	22 (21)
Dworkin et al[5]	50	1	6	7 (14)
Brooks and Enterline[6]	58	12	28	40 (68)
Shimm et al[7]	26	3	16	19 (73)
Moore and Wright[8]	36	5	31	36 (100)
Dragosics et al[9]	150	5	69	74 (50)
Berger et al[10]	23	3	12	15 (65)
Total	507	39	204	243 (48)

FCC, follicle center-cell lymphoma. This includes cases so designated as well as cleaved cells, large or small, centrocytes, large noncleaved cells and centroblasts.

A review of eight recent series of primary gastrointestinal lymphomas[3-10] (Table 3) revealed remarkably few tumors (39 of 507, 7.7%) judged to be follicle center-cell derived and to show at least a partial follicular pattern. A greater percentage (40.2%) were believed to represent tumors of follicle center-cell origin growing in a diffuse pattern. Thus, the collective view seems to be that some 48% of primary gastrointestinal lymphomas are of follicle center-cell lineage. Many of these reviews are heavily weighted towards high grade tumors, having specifically excluded pseudolymphomas, many if not most of which are really low grade lymphomas.[12,34] The inclusion of these low grade tumors would almost certainly increase the number of cases with a follicular histologic pattern for the reasons previously alluded to in this article. Nevertheless, based on the quoted reviews, a significant number of primary B cell gastrointestinal lymphomas should show bcl-2 gene rearrangement.

There has not been a previous investigation of bcl-2 gene rearrangements in a series of lymphomas arising from MALT, although rearrangement of the gene has been found in a single case of primary B cell gastric lymphoma.[35] We were unable to detect any bcl-2 gene rearrangement in our 21 cases of this type of lymphoma, even in those cases in which a follicular pattern was prominent. Compared with the high incidence of the bcl-2 gene rearrangements found in the follicular lymphomas, the absence of detectable alterations of the gene in the gastrointestinal MALT lymphomas may be evidence that the tumor cells or follicles of these two groups of the lymphoma are not identical at the genetic level. Our findings, therefore, support the previously made suggestion that lymphomas arising from MALT are not of follicle center-cell origin.[13]

Given that the incidence of bcl-2 gene rearrangement in high grade B cell tumors believed to be follicle center-cell derived is only 28%,[22] the significance of the absence of rearrangement in the five high grade cases included in this series is open to question. However, in at least some high grade primary B cell gastric lymphomas a minor component of low grade MALT lymphoma can be recognised (Chan JC, Isaacson PG, unpublished observations, 1989), suggesting a common cell lineage for both. This was true in three of the five high grade cases reported here.

Although four t(14:18) breakpoint cluster regions have been identified on chromosome 18, almost all the t(14:18) translocations so far analyzed at the molecular level fall within bcl-2 major or minor breakpoint regions.[22,31,32] Because bcl-2 gene rearrangement could not be demonstrated in our 21 cases of the lymphomas from MALT with the probes to these two regions, the t(14:18) translocation is unlikely to be a feature of this group of tumors. The immunoglobulin gene rearrangements found in these lymphomas may result from genetic abnormalities unique to the tumor cells, such as deletion of a specific region of the immunoglobulin gene or chromosomal translocation involving other known or unknown oncogenes. Further molecular analyses of this type of tumor exploring these possibilities are in progress.

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