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Rapid Communication

Different *bcl*-2 Protein Expression in High-grade B-cell Lymphomas Derived from Lymph Node or Mucosa-associated Lymphoid Tissue

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High-grade B-cell lymphomas, whether originated in a lymph node or in mucosa-associated lymphoid tissue (MALT), show similar morphologic traits, a fact that has fueled a long-running controversy about whether they represent different entities.[1–3] They differ, however, in that some high-grade MALT lymphomas show less aggressive clinical behavior, a focal low-grade component being identified in some of them. In a search for* bcl-*2 protein expression, we have found a significant difference between nodal (39/48) and MALT high-grade B-cell lymphoma (1/15) (P < 0.01).* Bcl-*2 gene product is an inner mitochondrial membrane protein able to give a survival advantage to B-cell lines by blocking programmed cell death.[4] This protein is usually expressed by memory or resting B cells, most activated B cells being* bcl-*2 negative, except in lymph–node-originated high-grade B-cell lymphomas, which appear to be mainly* bcl-*2 positive. Presence of* bcl-*2 protein in nodal large-cell lymphomas seems to be independent of a t(14;18) translocation,[5,6] only being found in 19 to 28% of these lymphomas, although it constitutes a definite difference between both tumors, suggesting the existence of different molecular genetic characteristics and pathogenesis, and is possibly related to the more aggressive clinical behavior of nodal high-grade tumors. (Am J Pathol 1991, 139:989–993)

High-grade B-cell lymphomas originating in mucosa-associated lymphoid tissue (MALT) are the most common type of lymphoma in mucosa,[1,7] being composed of large B cells with a diffuse pattern. These tumors are morphologically similar to B-cell high-grade lymphomas of nodal origin, usually being diagnosed as large B-cell lymphomas, of centroblastic or immunoblastic type. In spite of the morphologic similarities, most high-grade B-cell lymphomas originated in MALT have a more benign clinical course, it being possible in some of them to identify a peripheral remnant of low-grade B-cell lymphoma with lymphoepithelial lesions,[8,9] which suggests their origination in a previous low-grade tumour.

Bcl-2 oncogen, present in 18q21, is deregulated through juxtaposition with the immunoglobulin heavy chain locus in most follicular lymphomas.[10,11] The expression of the *bcl*-2 protein in nontumoral lymphoid tissue is mainly restricted to small lymphoid cells in a G0/G1 nonproliferative stage. In contrast with this finding, the expression of *bcl*-2 protein in tumoral tissue seems to be a frequent finding in germinal center originated B-cell lymphomas, having been found in a previous study in most follicular CB-CC lymphomas (37/43) as well as in B-cell high-grade lymphomas (11/14).[5]

The observation of absence of *bcl*-2 expression in some cases of MALT high-grade B-cell lymphomas prompted us to undertake a more exhaustive study, which aimed at the phenotypical characterization of the expression of *bcl*-2 protein in large B-cell lymphoma, of either nodal or MALT origin.

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Material and Methods

Tissue Samples

All cases studied are drawn from the record files of the Pathology Service in the Hospital Virgen de la Salud, Toledo.

Fifteen high-grade B-cell lymphomas of MALT origin were included in this study. All of them, at the moment of diagnosis, presented disease restricted to the gastrointestinal tract (stomach or intestine) and regional lymph nodes. Burkitt's lymphoma cases were excluded.

Forty-eight high-grade B-cell lymphomas of nodal origin, corresponding to consecutive cases of centroblastic, immunoblastic, or anaplastic large-cell lymphoma with available frozen tissue were also selected for the study. Only cases of well-proven lymph-node origin were included in this group.

Tissue Handling and bcl-2 Staining

Shortly after surgery, representative blocks of tissue were frozen in liquid nitrogen and stored at −70°C until use.

All cases were stained using a monoclonal antibody for the *bcl-2* protein (supplied by Dr. D. Y. Mason),[5] using the APAAP procedure[12] in frozen sections. Fisher's exact test was used to compare frequencies in 2-×-2 tables.

Results

High-grade B-cell Lymphomas

High-grade B-cell lymphomas of MALT origin were mainly of the classical or polymorphic centroblastic variant (12 cases), with one case of immunoblastic and two of anaplastic large-cell type.

The large tumoral cells were, in all cases except one, *bcl-2* negative. An internal control of the immunostaining was provided by the few small *bcl-2* positive lymphocytes present around small vessels or scattered between the tumoral cells (Figure 1).

Two of the cases studied contained a low-grade area composed of strands of small *bcl-2* positive B-cells disposed around *bcl-2* negative germinal centers in the periphery of the high-grade main component.

The *bcl-2* positive MALT lymphoma was morphologically classified as an anaplastic large cell lymphoma, B-cell type, showing strong cytoplasmic reactivity of the large pleomorphic cells with the *bcl-2* antibody.

Large-cell tumors of nodal origin were *bcl-2* positive in 39/48 cases (Table 1).

Discussion

Most cases of high-grade B-cell lymphomas originating in MALT are composed of centroblastlike (large noncleaved) B-cells, with a variable admixture of immunoblasts and pleomorphic multilobated large cells, being classified as monomorphic CB, polymorphic CB, or immunoblastic lymphoma in the Kiel classification. However, these tumors differ in other features from node-based high-grade B-cell lymphomas, such as their larger clear cytoplasm and more indolent clinical course.[7] A complementary finding is the presence in 30 to 40% of MALT high-grade lymphomas of a small-cell component with lymphoepithelial lesions, disposed peripherally to the main macroscopic lesion, suggesting that some of these high-grade B-cell lymphomas could originate in previous low-grade lymphomas,[8,9] as both components share the same light-chain restriction. Nevertheless, more definite proof, which would establish a clear difference between MALT and nodal high-grade B-cell lymphomas, is lacking.

Bcl-2 gene product is an inner mitochondrial membrane protein, able to give a survival advantage to B-cell lines by blocking programmed cell death and, synergically with the c-myc oncogen, to induce high-grade B-cell lymphomas in transgenic mice.[4,13]

In nontumoral lymphoid tissue, this protein is expressed in the cytoplasm of small lymphocytes present in peripheral blood or lymph node.[5,14] The activation of B-cells seems to correlate with the suppression of production of the *bcl-2* protein, germinal center B-cells being *bcl-2* negative.[5,15]

Double-staining studies using *bcl-2* and Ki67 monoclonal antibody have shown an exclusion of both antibodies, this finding constituting an additional proof that proliferating cells become *bcl-2* negative.[5]

The *bcl-2* gene is believed to be activated after a successful immunoglobulin gene rearrangement, enabling the cell to survive as a memory cell.

The *bcl-2* negativity of germinal center B cells is modified in follicular CB-CC lymphoma, in which tumoral cells are found to be positive in 90% of cases, irrespective of the presence of a t(14;18) translocation.[6] Our results also suggest that the *bcl-2* protein could be present in most (39/48) cases of node-based high-grade B-cell lymphomas of presumable germinal center origin, i.e., centroblastic or immunoblastic. This result contrasts sharply with that obtained in gastrointestinal B-cell high-grade tu-

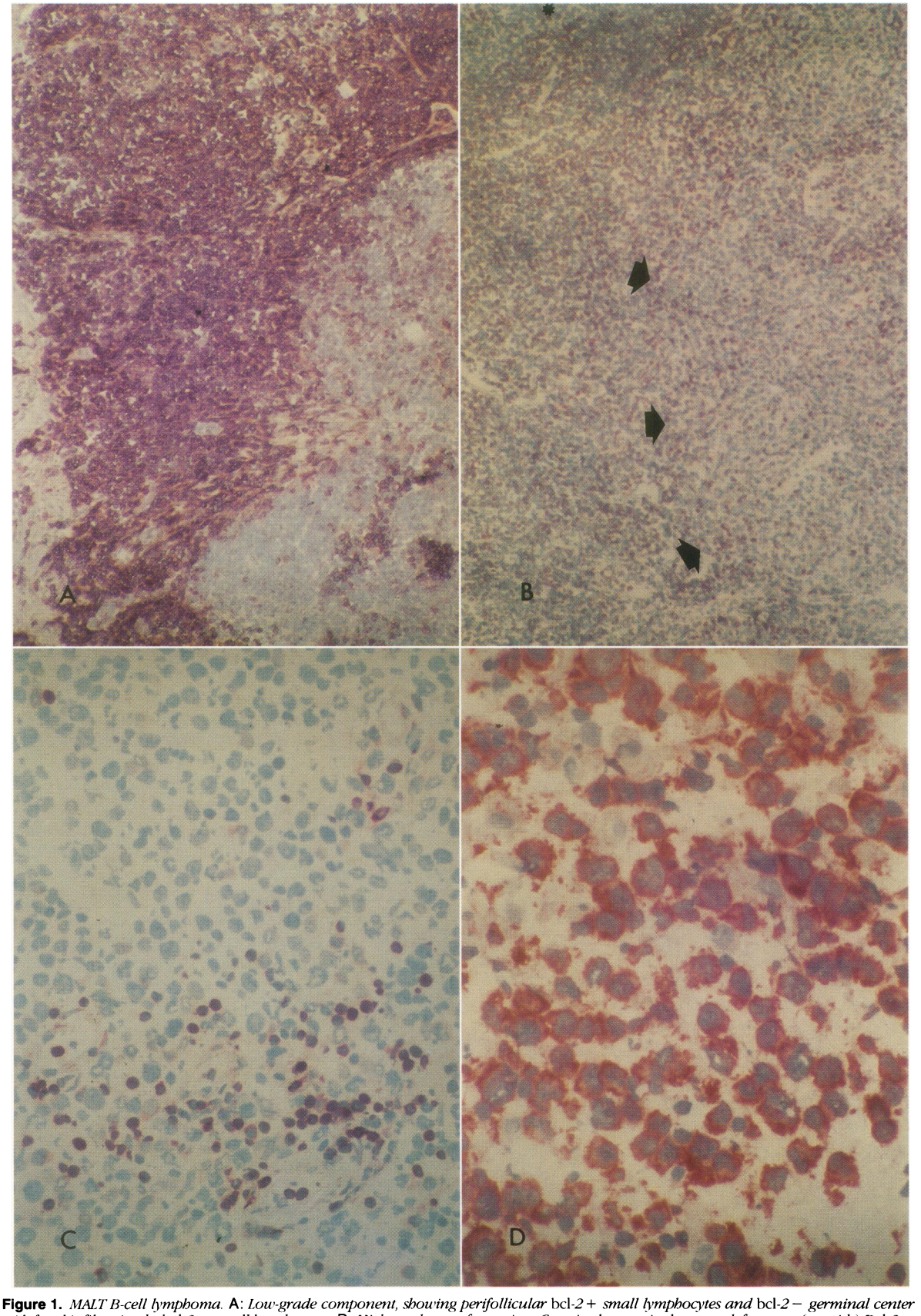

Figure 1. *MALT B-cell lymphoma.* **A**: *Low-grade component, showing perifollicular* bcl-2 + *small lymphocytes and* bcl-2 − *germinal center with focal infiltration by* bcl-2 + *small lymphocytes.* **B**: *High-grade transformation. Germinal center in the upper-left corner (asterisk).* Bcl-2 + *small-cell component at the left of the field transforming into* bcl-2-*large cell component at the center (arrows).* **C**: *High-grade tumor showing large* bcl-2-*cells and small* bcl-2 + *lymphocytes.* **D**: *Lymph-node originated large-cell lymphoma.* Bcl-2 *strong cytoplasmic reactivity.*

Table 1. Bcl-*2 Expression in Malt vs. Nodal High-grade B-cell Lymphomas* ($P < 0.01$)

	Total	*Bcl2* −	*Bcl2* +
Nodal			
Centroblastic	26	4	22
Immunoblastic	18	2	16
Anaplastic	4	3	1
Total	48	9	39
Gastrointestinal			
Centroblastic	12	12	0
Immunoblastic	1	1	0
Anaplastic	2	1	1
Total	15	14	1

mors, in which only 1/15 cases has been found to be *bcl-2* positive.

The results of the present study, as well as constituting a significant phenotypic feature useful for differential diagnosis, could also be related to the different clinical behavior of both types of tumor, since previous studies have suggested that *bcl-2* gene rearranged large-cell lymphomas have a poorer response to therapy than those in patients without *bcl-2* rearrangement.[16] In fact, *bcl-2* positive cells could constitute a reserve of resting B cells (as in follicular lymphoma), in G0 phase, insensitive to chemotherapy, which could explain the higher frequency of relapses in high-grade nodal lymphomas, in contrast with MALT origin tumors. In fact, the only *bcl-2* positive MALT high-grade B-cell lymphoma found in our series was also the only tumor that, after 50 months of complete remission, presented a relapse.[17]

Bcl-2 negativity in high-grade B-cell MALT lymphoma contrasts with the presence of the *bcl-2* protein in low-grade B-cell MALT lymphoma found in the two cases with adjacent low-grade component as well, and previously described by Isaacson et al.[18] This suggests that *bcl-2* protein disappeared during the transition from low-grade MALT lymphomas to high-grade tumors, an interpretation supported by the recent finding of Isaacson et al.[18] of absence of *bcl-2* expression in germinal centers colonized by tumoral cells since the germinal center is one of the environments where small tumoral *bcl-2* positive cells are transformed into large *bcl-2* negative cells.

The absence of the *bcl-2* protein in MALT lymphoma is in agreement with previous molecular studies that concluded that primary gastrointestinal lymphomas have different molecular genetic characteristics and pathogenesis, compared with node-based follicle–center cell lymphoma.[19–21] These differences could be partially explained by the suggested origin of MALT B-cell lymphomas in the marginal zone of the lymphoid follicles of the mucosae,[2] instead of the follicle–center origin of the nodal tumors of similar morphology, our findings supporting the previous hypothesis that large B-cell lymphomas in MALT could represent neoplasias of blastic variants of marginal zone B cells.[8]

The frequent presence of *bcl-2* protein in nodal high-grade lymphomas (39/48) cannot only be explained by the t(14;18) translocation, present only in 19 to 28%[2,10,16,23–25] of nodal high-grade lymphomas, which suggests the necessity of searching for a different molecular mechanism of *bcl-2* activation.[6]

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