Expression of the bcl-2 protein in B cell lymphomas arising from mucosa associated lymphoid tissue

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Abstract

Aim—To determine whether lymphomas arising from mucosa associated lymphoid tissue (MALT) express the bcl-2 protein.

Methods—Forty two cases of MALT B cell lymphomas, 20 low grade neoplasms and 22 high grade tumours, were studied. Immunohistological staining was performed on paraffin wax embedded tissue using a monoclonal antibody specific for the bcl-2 protein.

Results—All of the low grade lymphomas gave positive results on staining, with clear cytoplasmic labelling for bcl-2 protein in the small neoplastic cells, some of which formed characteristic lymphoepithelial lesions. A striking feature was that larger bcl-2 negative cells were observed in nine of these tumours. They were either scattered singly among the small neoplastic cells or formed small clusters, suggesting that they could represent early areas of transformation to high grade neoplasia. Germinal centres in the vicinity of the tumours lacked bcl-2 protein and hence contrasted clearly with the neoplastic cells. In some cases this permitted germinal centres, which were not obvious on conventional histological staining, to be recognised. In 20 of the 22 cases of high grade B cell lymphoma the large neoplastic cells were bcl-2 negative; the remaining two cases, however, con-

tained a proportion of large neoplastic bcl-2 positive cells. In four of the 22 cases of high grade tumours a low grade component was found which expressed bcl-2 in all cases.

Conclusion—Bcl-2 protein is expressed in low grade, but not in most high grade, MALT lymphomas. In view of recent data indicating that most high grade nodal lymphomas express bcl-2, these findings suggest that MALT lymphomas may regulate bcl-2 gene expression differently to nodal lymphomas.

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Keywords: Bcl-2 protein expression, MALT lymphoma.

Low grade B cell lymphomas originating in mucosa associated lymphoid tissue (MALT)¹⁻⁶ account for a large proportion of extranodal low grade B cell lymphomas. They arise in the gastrointestinal tract but also in other organs which contain MALT, including the lungs and the thyroid and salivary glands.⁴ These lymphomas often take the form of a diffuse infiltration of neoplastic cells, possessing a centrocyte-like morphology, often associated with prominent reactive germinal centres and lymphoepithelial lesions. Low grade B cell MALT lymphomas are characterised by an indolent clinical course and prolonged localisation of the tumour to the original site without dissemination.⁴

The bcl-2 protein is mainly expressed in the inner mitochondrial membrane and can extend cell survival by blocking programmed cell death ("apoptosis").⁷ It is absent from reactive germinal centres but is often expressed in neoplastic germinal centres in follicular B cell lymphomas,⁸ usually in association with the 14;18 chromosomal translocation. The expression of bcl-2 protein has also been reported in many types of nodal non-Hodgkin's lymphomas, independently of the presence of the 14;18 translocation.^{9,10} However, the absence of bcl-2 expression in most cases of MALT high grade B cell lymphoma has been reported¹¹ and this prompted us to compare bcl-2 protein expression in low and high grade MALT lymphomas. As labelling was performed on paraffin wax embedded tissue, we were also able to assess the relation between bcl-2 expression and cell morphology.

Table 1 Bcl-2 reactivity of MALT lymphomas: Low grade MALT lymphomas

Histology				
Site	Small neoplastic cells	Centroblasts or immunoblasts	Reactive follicles	Bcl-2 staining Small neoplastic cells
Stomach	Centrocyte-like	Absent	Present	+++
Stomach	Centrocyte-like	Absent	Present	+++
Stomach	Centrocyte-like	Clusters	Present	++
Stomach	Centrocyte-like	Rare	Present	+++
Stomach	Centrocyte-like	Clusters	Absent	+++
Stomach	Centrocyte-like	Absent	Present	+++
Stomach	Lymphoplasmacytoid	Rare	Present	+++
Stomach	Lymphoplasmacytoid	Rare	Present	+++
Stomach	Lymphoplasmacytoid	Rare	Present	+++
Stomach	Lymphoplasmacytoid	Rare	Present	++
Colon	Centrocyte-like	Absent	Present	+++
Thyroid	Centrocyte-like	Rare	Absent	+++
Thyroid	Lymphoplasmacytic	Absent	Absent	++
Lung	Centrocyte-like	Rare	Present	++
Lung	Centrocyte-like	Absent	Present	+++
Lung	Centrocyte-like	Absent	Absent	+++
Lung	Centrocyte-like	Absent	Present	++
Lung	Centrocyte-like	Absent	Absent	++
Salivary gland	Centrocyte-like	Absent	Present	+++
Salivary gland	Centrocyte-like	Absent	Present	+++

Staining intensity for bcl-2 is indicated on a scale from negative (-) to strongly positive (+++).

Table 2 Bcl-2 reactivity of MALT lymphomas: High grade MALT lymphomas associated with a low grade component

Histology				Bcl-2 staining	
Site	Small neoplastic cells	Large neoplastic cells	Reactive follicles	Small neoplastic cells	Large neoplastic cells
Stomach	Centrocyte-like	Centroblast-like	Present	++	-
Stomach	Lymphoplasmacytoid	Immunoblastic	Present	++	-*
Ileum	Centrocyte-like	Centroblast-like	Present	++	-*
Thyroid	Lymphoplasmacytoid	Immunoblastic	Present	-*	-

*Rare positive cells.
Staining intensity for bcl-2 is indicated on a scale from negative (-) to strongly positive (+++).

Table 3 Bcl-2 reactivity of MALT lymphomas: High grade MALT lymphomas

Histology				Bcl-2 staining	
Site	Small neoplastic cells	Large neoplastic cells	Reactive follicles	Small neoplastic cells	Large neoplastic cells
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Stomach	Absent	Centroblast-like	Present	-	-
Colon	Absent	Centroblast-like	Present	-	-
Colon	Absent	Centroblast-like	Present	-	-
Ileum	Absent	Centroblast-like	Present	-	-
Ileum	Absent	Centroblast-like	Present	-	-
Ileum	Absent	Centroblast-like	Present	-	-
Ileum	Absent	Centroblast-like	Present	-	-

Staining intensity for bcl-2 is indicated on a scale from negative (-) to strongly positive (+++).

Methods

All tissue specimens were obtained following surgical resection. The material consisted of 46 cases of B cell lymphoma of MALT origin, including gastric, colorectal, ileal, pulmonary, salivary gland, and thyroid gland lymphomas (tables 1-3). The B cell origin of these tumours was assessed by their reactivity with the L26 (CD20) monoclonal antibody or their monotypic immunoglobulin expression on frozen sections. All samples had been fixed in Bouin's fixative or formalin and embedded

Figure 1 Pulmonary low grade B cell MALT lymphoma. Surface epithelium invaded by centrocyte-like lymphoma cells stained for bcl-2.

in paraffin wax. These sections were then cut at 4 µm and stained with haematoxylin and eosin and Giemsa stains. Immunostaining was performed on deparaffinised sections by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique,¹² using a monoclonal antibody (bcl-2/100) generated against a synthetic peptide.¹⁰ Of the 46 cases, four were excluded after initial review because of unsatisfactory bcl-2 staining (no staining of any cells, including reactive cells), leaving 42 in which there was clear staining of neoplastic and/or normal cells. Twenty of these cases were classified histologically as low grade and 22 as high grade tumours. In four of the 22 high grade tumours a low grade component was found.

Results

In all 20 cases (table 1) the neoplastic cells were bcl-2 positive (figs 1 and 2). In 15 cases the tumour cells were of centrocyte-like morphology. In four cases most of the tumour cells were of lymphoplasmacytoid morphology and the remaining case was a lymphoplasmacytic tumour. In 14 cases the bcl-2 positive neoplastic cells were more strongly stained than reactive mantle zone lymphocytes, while in the remaining six cases their labelling was of comparable intensity. Lymphoepithelial lesions were seen in many cases, comprising labelled centrocyte-like cells infiltrating surface epithelium (fig 1). In seven of the 20 cases a small number of large centroblast-like bcl-2 negative neoplastic cells were scattered among the centrocyte-like neoplastic cells, and in two further cases small clusters of such cells could be identified morphologically amongst the smaller neoplastic cells (fig 3).

In 15 cases reactive secondary follicles were present in the vicinity of the neoplasm, and in each instance the germinal centre cells were bcl-2 negative (fig 2). In some cases this enabled residual germinal centres to be recognised which were not discernible on conventional histological staining.

The 22 cases in this category were mainly composed of large neoplastic cells resembling centroblasts (21 cases) or immunoblasts (one case). In 20 of the 22 cases of high grade B cell lymphomas the large neoplastic cells were bcl-2 negative (fig 4), whereas the remaining two cases contained a few large bcl-2 positive cells. In four of the 22 cases of high grade tumours a low grade component was found, and three of these expressed the bcl-2 protein (fig 5). In the remaining case most of the neoplastic cells with lymphoplasmacytoid morphology were bcl-2 negative. In each negative case small reactive lymphocytes scattered amongst the neoplastic cells provided an internal positive control (fig 4).

Discussion

The present study indicates that the bcl-2 protein is expressed by the neoplastic cells in all low grade B cell lymphomas of MALT origin. Although rearrangements of the

Figure 2 Gastric low grade B cell MALT lymphoma. Centrocyte-like lymphoma cells surrounding a negative reactive follicle.

Figure 3 Gastric low grade B cell MALT lymphoma. Centrocyte-like lymphoma cells stained for bcl-2 contrasting with scattered centroblastic and immunoblastic large negative cells.

Figure 4 Gastric high grade B cell lymphoma. Neoplastic large cells negative for bcl-2, contrasting with the presence of bcl-2 positive small normal lymphocytes.

Figure 5 High grade MALT lymphoma associated with low grade B cell MALT lymphoma. Centrocyte-like lymphoma cells stained for bcl-2 surrounding a reactive germinal centre. ○ = A bcl-2 negative area composed of large "transformed" lymphoid cells.

bcl-2 gene have been rarely detected in lymphomas of MALT origin,¹³⁻¹⁶ expression of the *bcl-2* protein in MALT lymphomas is not unexpected, as *bcl-2* expression has been reported in many node based non-Hodgkin's lymphomas which lack t(14;18). Bcl-2 protein expression also occurs in Reed-Sternberg cells in some cases of Hodgkin's disease as well as in normal B and T lymphocytes.^{9 10 17 18}

Occasionally, the diffuse infiltrate of small centrocyte-like cells, which tends to invade mucosal epithelium to form characteristic lymphoepithelial lesions in low grade B cell MALT lymphomas, may transform into a high grade large cell neoplasm.¹⁹ It was therefore of interest that large *bcl-2* negative cells were seen in the present series, either scattered or in small clusters amongst small *bcl-2* positive cells in nine of 20 of the low grade tumours. This indicates that low grade MALT lymphomas have a tendency to lose *bcl-2* protein when they progress to high grade tumours. A parallel can be drawn with our previous findings in centroblastic/centrocytic follicular lymphomas, in which large non-cleaved cells (centroblasts) may be negative for *bcl-2* while small cleaved cells (centrocytes) express this protein strongly.⁸

The tendency of high grade MALT lymphomas to lose *bcl-2* protein expression has been confirmed in the present series as in 20 the large neoplastic cells lacked *bcl-2* and in the two remaining cases only a proportion of large tumour cells expressed *bcl-2*. This contrasts with the *bcl-2* positive results reported in most cases of nodal large B cell

lymphoma^{11 18} but is in keeping with the previous observation that most large cell MALT lymphomas are bcl-2 negative.^{11 20} This suggests that MALT lymphomas differ from nodal lymphomas in the regulation of bcl-2 gene expression.¹⁴ The absence of detectable bcl-2 protein in most high grade MALT lymphomas could reflect a breakdown in bcl-2 gene expression at the transcriptional or the post-transcriptional level. The possibility of post-transcriptional breakdown in bcl-2 gene expression has been considered based on discordant bcl-2 messenger RNA (mRNA) and bcl-2 protein expression in reactive lymph nodes.^{20 21} In addition, stimulatory and inhibitory mechanisms of bcl-2 gene expression at the transcriptional and post-transcriptional level have been described in human proliferating lymphocytes and lymphonodal lymphomas.^{22 23} Further studies showed that proliferating normal lymphoid cells fail to express the bcl-2 protein.¹⁰ Therefore, the absence of bcl-2 protein expression in most high grade MALT lymphomas may be related to their high rate of proliferation. In this respect Isaacson *et al*²⁴ have shown that colonised follicles in MALT lymphomas are bcl-2 negative. This finding may be related to the high proliferation rate in colonised follicles, or to physiological down-regulation of bcl-2 in the follicular environment.²⁴

The clinical and prognostic importance of the absence of bcl-2 protein expression in high grade MALT lymphomas when compared with high grade lymphonodal lymphomas has not been established. However, high grade MALT lymphomas exhibit a better prognosis than high grade lymphonodal lymphomas.²⁵ This might be related to findings that bcl-2 positive high grade lymphomas have a shorter disease free survival than bcl-2 negative ones¹⁸ and that a B leukaemic cell line with elevated bcl-2 protein levels exhibits a strikingly long survival when cultured in the presence of antineoplastic drugs.²⁶

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