

Indolent lymphoma: the pathologist's viewpoint

S. A. Pileri^{1*}, P. L. Zinzani², P. Went³, A. Pileri Jr¹ & M. Bendandi^{4†}

¹Pathologic Anatomy and Unit of Haematopathology, Institute of Haematology and Clinical Oncology 'L. & A. Seràgnoli', Bologna University; ²Lymphoma Unit, Institute of Haematology and Clinical Oncology 'L. & A. Seràgnoli', Bologna University, Bologna, Italy; ³Institute of Pathology, University Hospital Basel, Basel, Switzerland; ⁴Department of Haematology and Cell Therapy Area, University Clinic, University of Navarra, Pamplona, Spain

Received 30 April 2003; accepted 7 August 2003

Indolent lymphomas have recently been the object of numerous studies, which have focused on new aspects relevant both for the better comprehension of their histogenesis and the identification of new therapeutic strategies. As marginal-zone lymphoma (MZL) represents the category of indolent lymphomas that has obtained more benefit from such an approach, the authors focused on the most recent achievements and not yet solved controversies in this area. In spite of their postulated common derivation, the three categories of MZL of the WHO Classification appear dissimilar. In fact, they show significant molecular differences among them as well as a certain heterogeneity within each group. By no means, there is a cogent need of more refined tools to revise these neoplasms and to produce a more rational grouping. The recent identification of the IRTA gene family corresponding to IG-like receptors differentially expressed in B-cells might contribute to their better understanding.

Key words: extranodal marginal zone lymphoma, indolent lymphoma, lymphoma classification, nodal marginal zone lymphoma, primary splenic marginal zone lymphoma

Introduction

In 1994, the members of the International Lymphoma Study Group (ILSG) proposed the Revised European American Lymphoma (REAL) classification [1], aiming to overcome the different approaches (e.g. the Kiel classification [2, 3] and working formulation [4]) employed in Europe and the USA, which hampered the comparison among clinico-pathological trials. The proposal of the REAL classification was followed by a validation study, carried out by pathologists external to the ILSG and based on a series of cases collected worldwide, which showed that the ILSG scheme was superior to both the Kiel classification and working formulation in terms of interpersonal and intrapersonal reproducibility [5]. More recently, the World Health Organisation (WHO) has adopted the REAL classification as a model for the categorisation of all the tumours of the lymphoid and haematopoietic tissues [6].

The REAL/WHO classification [1, 6] consists of a list of distinct entities, which are defined by the amalgamation of morphology, phenotype, genotype and clinical findings, and can be updated on the basis of new evidence emerging from the literature. Conversely to previous schemes, the REAL/WHO classification does not provide grades of malignancy, as the clinical behaviour and response to therapy are not influenced by the cell size and number of mitotic figures, as postulated in the Kiel classification

and working formulation, but depend on the category the tumour belongs to and within each category on a series of biological mechanisms, which are at work differently in each individual patient. On one hand, this explains the artificiality of certain distinctions of the past, such as the ones between low- and high-grade peripheral T-cell lymphomas, all types of T-cell tumour showing a poor prognosis with the exception of mycosis fungoides and ALK-positive anaplastic large cell lymphoma. On the other hand, such an approach has favoured research activity to better define the risk factors within each lymphoma category, that has found its cutting edge in the tissue micro-array and gene expression profiling techniques.

In 1995, Dan Longo [7] proposed the usage of some terms such as indolent, aggressive and very aggressive lymphoma, which refer to the natural history of the disease irrespective of its response to therapy. Although they are not included in the REAL/WHO classification, these terms are commonly used in daily practice. In particular, the label 'indolent' lymphoma applies to lymphoid tumours with a survival measurable in years, irrespective of whether or not any therapy is given. These lymphoproliferative disorders have variable clinical presentations. Some are constantly systemic diseases, often with leukaemic manifestations. Others have an extranodal primary presentation and can remain localised for long periods, even in the absence of any therapy. Yet others correspond to tumours with nodal presentation, which can have widespread immune system involvement at the time of diagnosis. This has led to the basic distinction of three fundamental subtypes of indolent lymphoma: disseminated leukaemias/lymphomas, extranodal forms and nodal ones (Table 1). Interestingly, all these neoplasms are derived from the B-cell system, except T large granular lymphocyte leukaemia. In spite of the fact that they

*Correspondence to: Prof. S. A. Pileri, Cattedra di Anatomia Patologica ed Unità Operativa di Ematopatologia, Istituto di Ematologia e Oncologia Medica 'L. & A. Seràgnoli', Policlinico S. Orsola, Università di Bologna, Via Massarenti 9, 40138 Bologna, Italy. Tel: +39-051-6363044; Fax: +39-051-6363606; E-mail: pileri@almadns.unibo.it
†Leukemia and Lymphoma Society Scholar in Clinical Research.

Table 1. List of indolent lymphomas

Disseminated lymphomas/leukaemias
B-cell chronic lymphocytic leukaemia
Lymphoplasmacytic lymphoma
Hairy cell leukaemia
Splenic marginal zone B-cell lymphoma (\pm villous lymphocytes)
Plasma cell myeloma/plasmacytoma
T large granular lymphocyte leukaemia
Extranodal lymphomas
Extranodal marginal zone B-cell lymphoma of MALT type
Nodal lymphomas
Small lymphocytic lymphoma
Follicular lymphoma
Nodal marginal zone B-cell lymphoma (\pm monocytoid B-cells)

MALT, mucosa-associated lymphoid tissue.

correspond to entities widely acknowledged in the literature, most of them have recently been the object of challenging molecular studies, which have focused on new aspects relevant both for the better understanding of their histogenesis and the identification of new therapeutic strategies. As marginal zone lymphoma (MZL) represents the category of ‘indolent lymphomas’ that has obtained most benefit from such an approach [8], this review focuses on the most recent achievements and as yet unsolved controversies in this area.

Marginal zone lymphoma

The REAL classification [1] listed three types of MZL: extranodal, nodal and primary splenic. The former was regarded as an accepted entity, while the latter two were considered as provisional entities. The WHO classification [6] listed the three forms as accepted entities. However, there are still many uncertainties as to whether or not they represent a homogeneous group of tumours.

Normal marginal zone B-cell compartment

The marginal zone (MZ) corresponds to the outer part of secondary follicles (for a comprehensive review of the topic see Kurtin [9]). It is well developed and easily recognisable in the spleen, intra-abdominal lymph nodes and mucosa-associated lymphoid tissue (MALT). The latter corresponds to Peyer patches physiologically present at birth (native MALT) or develops during life at different anatomic sites, including the stomach, thyroid, salivary gland, lung and skin, because of a chronic inflammation sustained by an infective agent and/or an auto-immune condition (acquired MALT). Most MZ cells express CD19 and CD20 and have the phenotypic profile of memory B-cells. Thus, they are strongly positive for immunoglobulin (Ig) of the M class, IgG or IgA, only a small subpopulation exhibiting weak IgD staining. They also express CD21, CD27 and Bcl-2 protein, but are negative for CD5, CD10, CD23, CD43, CD75 and Ki-B3 (an antibody that recog-

nises a glycosilation-independent epitope of CD45RA, expressed by B-lymphocytes that are immunocompetent, but have not yet responded to antigens). A subset of splenic MZ cells, however, shares phenotypic features with mantle B-elements by showing positivity for IgM, IgD and Ki-B3, and negativity for CD21 and CD27. On molecular grounds, micro-dissection PCR studies have shown that most splenic MZ B-cells contain point mutations of the Ig genes at frequencies found in post-follicular memory B-elements. However, a small subset of the same MZ B-cells displays a low load of Ig gene point mutations, as usually found in mantle B-cells. Therefore, although belonging to the same anatomic compartment, splenic MZ B-elements do show a certain phenotypic and molecular variability, the vast majority of them being likely part of the recirculating memory B-cell pool. Interestingly, splenic MZ B-cells can bind polysaccharide antigens with one of two results, depending on the follicle microenvironment. First, they can migrate into the germinal centres (GC) and present the antigen to GC B-cells. If follicular dendritic cells have surface Ig that binds to the presented antigen, they proliferate and give rise to the GC cell reaction. Second, antigen in association with cytokines released by T-cells can rapidly induce differentiation of MZ B-cells into plasma cells, which in turn synthesise and release antigen-specific Ig.

It has repeatedly been reported in the literature that ‘monocytoid B-cells’ belong to the MZ B-cell compartment. These elements are characterised by medium size, distinct cellular contours, clear cytoplasm, variably shaped nuclei, and inconspicuous nucleoli [10]. They are commonly seen in lymph nodes during the course of different types of lymphadenitis and most often form clusters within or around sinuses and in the interfollicular areas, although they occasionally surround benign follicles to produce a marginal zone pattern [11]. In spite of the common belief that they belong to the MZ cell pool, which is probably based on their usual IgG expression, no firm proof has been provided yet that ‘monocytoid B-cells’ have post-GC cell derivation [12]. In fact, immunophenotypic analysis reveals positivity for Ki-B3/CD45RA and CD75 and negativity for CD21 and CD27 [9]. In addition, molecular studies display regular lack of somatic mutations of IgV_H genes as observed in naïve B-elements.

Extranodal marginal zone lymphoma

Extranodal MZL is thought to stem from MZ cells of MALT [13–17]. In the REAL/WHO classification [1, 6], by definition the term extranodal MZL is restricted to tumours consisting of small elements provided with centrocyte-like or monocytoid morphology and associated or not with plasmacytoid differentiation, which resemble normal MALT MZ cells and share with them phenotypic and molecular characteristics, including the IRTA-1 gene expression (B. Falini, personal communication). According to the original description, these neoplasms are also called MALT lymphomas.

Because of its high prevalence and clinical relevance, MZL MALT lymphoma of the stomach has been the object of recent research, which led to better understanding of its characteristics, including lympho-epithelial lesion formation, multicentricity, pathogenetic relationships to *Helicobacter pylori* (HP) infection

and susceptibility to antibiotics [17, 18]. Following preliminary reports which suggested that most gastric MZLs could regress following HP eradication [19–21], it is now clear that this event occurs in only 50–55% of cases [17, 18]. Interestingly, besides the regression of a gastric tumour, antibiotic therapy may at times produce disappearance of a contemporary extranodal MZL at another anatomic site: in one of these cases, a clonal relationship between the two tumours has been documented in the literature [22]. Ultrasound endoscopy has shown that regression is unlikely in cases with infiltration of the muscularis propria or entire gastric wall, as well as in patients with peri-gastric lymph node involvement [23].

Cytogenetic and molecular studies have recently shown that extranodal MZL is characterised by the occurrence of different chromosomal aberrations, which influence its invasive potential and possible response to therapy. In particular, three chromosomal aberrations have been detected, t(1;14), t(11;18) and t(14;18), which are possibly involved in the process of lymphomagenesis and affect the course of the disease [24]. t(1;14)(p22;q32) is exceedingly rare and causes transfer of the *BCL10* gene close to the Ig enhancer on chromosome 14, thus causing overexpression of the corresponding product that accumulates at the nuclear level, and may be provided with oncogenic activity [25–28]. t(14;18)(q21;q21) is detected in 30–35% of gastric MZLs and produces the formation of the fusion gene *API2-MALT1* [29–39]. Interestingly, this aberration, which can be reliably detected in routine material by appropriate PCR studies [32, 37], is also associated with BCL10 protein accumulation within the nucleus of neoplastic cells [25, 26, 28, 31, 33]. The explanation of how the same phenomenon can be pursued by seemingly unrelated translocations lays in the observation that under physiological conditions BCL10 and MALT1 products form a strong and specific complex. In particular, *BCL10* mediates the oligomerisation of the MALT1 caspase-like domain with subsequent activation of the IKK complex through an unknown mechanism, setting in motion a cascade of events leading to NF- κ B induction [26, 33]. Furthermore, the API2-MALT1 fusion protein itself seems to strongly activate NF- κ B and to show dependence upon the same downstream signalling pathway [26, 33]. Thus, both the BCL10.MALT1 complex and API2-MALT1 fusion protein might activate a common route that originates with the oligomerisation-dependent activation of the MALT1 caspase-like domain and leads to resistance to antibiotic therapy [30, 37]. Notably, besides antibiotic-resistance, t(11;18) is characterised by a higher potential of local infiltration and distance spread, as well as by the lack of both other chromosomal abnormalities and progression to a more aggressive tumour [29, 31, 35, 36]. Epidemiological studies have recently shown that the translocation is even more frequent in the lung than in the stomach [34, 39] and is found also in approximately one-half of the rare examples of gastric HP-negative MZL [38], thus further supporting the concept that tumours carrying t(11;18) do not need HP stimulation for their growth and maintenance. t(14;18)(q32;q21) has been the subject of much debate in the literature. In fact, some authors thought that the translocation at times found in extranodal MZLs could correspond to the one typically found in follicular lymphoma (FL) and involving the *BCL2* gene [1, 6]. In

reality, the t(14;18) of MZLs does not affect *BCL2*, but does affect *MALT1* by possibly following the same pathogenetic pathway as t(1;14) and t(11;18) [40, 41]. According to the few data in the literature, the translocation involving the Ig enhancer and *MALT1* gene seems to be more frequent at anatomic sites other than the gastrointestinal tract and, like t(1;14), to occur either as the sole genetic abnormality or in conjunction with trisomy 3 and/or 18 [40]. It should always be distinguished from the translocation involving the *BCL2* gene by FISH and/or PCR analysis, also in the light of the fact that FL may at times show prominent MZ differentiation [42–45], thus blurring the morphological borders between the two tumours. Features of MZ differentiation may also occur in mantle cell lymphoma (MCL) [46]: under these circumstances, the detection of t(11;14) and cyclin D1 overexpression along with the lack of t(1;14), t(11;18) or t(14;18) does allow firm distinction of this aggressive neoplasm from MZL.

Besides chromosomal translocations, two other issues have gained attention in the field of MZLs: one of these pertains all tumours irrespective of their primary site, while the other is strictly related to gastric forms. In particular, the former issue refers to the onset of a large B-cell lymphoma at an anatomic site containing native or acquired MALT. Several authors have named this tumour ‘high-grade MZ/MALT lymphoma’. The latter term is not included in the REAL/WHO classification, because: the label ‘MALT’ lymphoma is by definition restricted to neoplasms showing small cell size and derivation from MALT MZ elements; there is no evidence that a large B-cell lymphoma occurring *de novo* at a MALT site is derived from MZ cells; and the clonal relationship between a MZL and a large B-cell neoplasm simultaneously detected in the same organ should be proven molecularly, as the latter can represent the blastic phase of the former, but might also develop as a second unrelated neoplasm [1, 6]. In the few cases in which a clonal progression from indolent to aggressive lymphoma has been proven, a loss of expression of $\alpha 4\beta 7$ integrin and L-selectin has been recorded and supposed to take part in the transforming event [47]. The other issue deals with the response to antibiotic therapy and follow-up of patients with gastric MZL and HP infection. First, morphology represents the most effective tool to assess lymphoma regression, as PCR studies can show a monoclonal signal even years following therapy in the absence of any sign of disease relapse [48–52]. Second, the monitoring of these patients should be performed according to clear-cut guidelines such as the ones proposed by the IELSG: the first biopsy should be performed 1 month after the end of antibiotic treatment to assess HP eradication, a second exam should be carried out 3 months later by taking multiple samples from the area where the diagnosis of lymphoma was originally made and at distance sites, in order to evaluate the tumour response [52]. Each sample should be histologically analysed at different levels to be sure that foci of the disease are not neglected. At present, microscopic examination is carried out according to the so-called Wotherspoon’s score [53]. However, there are several attempts underway to propose a monitoring approach more practical and reproducible than the one described originally by Wotherspoon (A. Wotherspoon and C. Copie-Bergmann, personal communication). In principle, in case of no response to eradicating therapy, the bioptic material should

be analysed on molecular grounds in order to check the possible occurrence of chromosomal aberrations, which prevent the successful application of antibiotics, thus leading to different options (such as chemotherapy, surgery, radiotherapy and/or immunotherapy). On the other hand, cases which do show a significant improvement after antibiotic therapy should be studied periodically over an indefinite period in order to evaluate the maintenance of disease remission or possible lymphomatous relapse or progression.

Nodal marginal zone lymphoma

In the WHO classification, nodal marginal zone lymphoma (NMZL) is defined as a primary nodal B-cell neoplasm that morphologically resembles lymph nodes involved by MZ lymphoma of the extranodal or splenic type, but without evidence of extranodal or splenic disease. According to this statement, one should conclude that the terms extranodal, nodal and splenic MZL refer to different clinical presentations of the same disease and the diagnosis of NMZL is based on the exclusion of a primary extranodal or splenic tumour [6]. Several reports have underlined that there are instead significant differences among the three neoplasms.

On clinical grounds, NMZL is more aggressive than the extranodal and splenic forms [54–56]. For example, it shows a higher incidence of advanced stage disease and lower 5-year overall and disease-free survival [54, 55]. In addition, 10–20% of cases tend to transform into a diffuse large B-cell lymphoma (DLBCL) [55]. At light microscopy, although centrocyte-like morphology can at times be observed, most cases display a distinct ‘monocytoid’ appearance [1, 6, 55]. Because of this, the tumour was originally termed ‘monocytoid B-cell lymphoma’ [55]. Accordingly, phenotypic analysis reveals common expression of Ki-B3/CD45RA and CD75 and negativity for CD21 and CD27, thus differentiating the nodal form from the splenic and extranodal forms [9]. Molecular studies further strengthen the existence of significant differences among the three tumours. First, t(11;18) has never been detected in NMZL [34]. Second, the analysis of IgV_H gene clearly shows that some NMZLs carry somatic mutations, while others do not, thus suggesting derivation from post-GC and virgin B-cells, respectively [56, 57]. By contrast, the occurrence of ongoing mutations represents a more controversial issue, as this might reflect the inclusion of FLs with MZ differentiation within this category [57]. Interestingly, among mutated cases the usage of specific IgV_H gene segments seems to occur frequently and to discriminate between hepatitis C virus (HCV)-positive and -negative patients [58]. In the former, there is preferential usage of a VH1-69 segment with similar CDR3s, thus suggesting the presence of a common antigen, probably a HCV antigen epitope, involved in B-cell selection, while in the latter the use of a VH4-34 segment might be related to the role of yet unknown B-cell superantigen(s) in the selection of tumour B-cell precursors. Finally, none of the translocations characteristically recorded in SMZL, e.g. del(7q), del(13q14) and del(10)(q22,q24), has so far been detected in NMZL, thus further challenging the concept that the three forms of MZL included in the WHO classification share a common histogenesis [59, 60].

Splenic marginal zone lymphoma

Splenic marginal zone lymphoma (SMZL) has been the object of numerous reports in the literature. It is generally characterised by splenomegaly and leukaemic spread, although at times cases with disseminated disease or exclusively leukaemic presentation have been described [56]. In about half of cases, circulating neoplastic cells display cytoplasmic villous projections [1, 6, 56], which justify the term ‘splenic lymphoma with villous lymphocytes’ (SLVL) used in former studies [61]. Infiltration of the bone marrow, which is recorded in most if not all patients, has been claimed to be limited to the sinuses: however, this finding is neither specific nor pathognomonic [56, 62, 63]. In fact, it can occur in other types of malignant lymphoma and in SMZL it does not represent the only type of bone marrow involvement, nodular and/or interstitial components being frequently encountered. No consensus has been achieved yet on the optimal therapeutic approach. With only a few exceptions [64], authors agree on the fact that subjects who underwent splenectomy fare better, thus suggesting that splenectomy may be the first-line treatment choice [65–68]. According to reported data, it can be delayed until the occurrence of symptoms or cytopenia and seems to be *per se* sufficient for correcting cytopenic manifestations, improving quality of life and increasing survival (with median values of between 9 and 13 years) [56, 68]. The utility of alternative or complementary approaches, such as chemotherapy, radiotherapy or the employment of humanised monoclonal antibodies targeted against B-cell-associated antigens, should be clarified in prospective trials [69]. On practical grounds, several adverse prognostic predictors have been quoted in the literature, including haemolytic anaemia, immune thrombocytopenia, M-component in the serum, elevated β 2-microglobulin level, leucocyte count >20 000/ μ l, lymphocytes >9000/ μ l, and p53 overexpression by neoplastic cells [56, 65–68]. Progression to DLBCL has been recorded in about 10% of patients [70].

The analysis of a large series of cases has recently shown that some tumours diagnosed as SMZL express CD5 and cyclin D1 and carry t(11;14) [56]. It is still unclear whether these cases represent examples of mantle-cell lymphoma with marginal zone differentiation. We have collected three neoplasms with these phenotypic and cytogenetic characteristics that showed the typical morphology of SMZL (unpublished observation): they consisted of medium-sized elements with clear cytoplasm and oval or kidney-shaped nuclei, which grew around Malpighi’s corpuscle with discrete diffusion through the splenic sinuses. The patients had villous circulating lymphocytes and bone marrow sinusoidal infiltration in the absence of other organ involvement. They all underwent splenectomy and did not receive further therapies. Two to 3 years after diagnosis, the patients are alive and well without signs of disease progression. The collection of further such cases is mandatory to assess which category of malignant lymphoma they belong to.

Cytogenetics and molecular studies have shown that SMZL is a heterogeneous tumour [56, 59, 60, 71]. In particular, Solé et al. have proposed the distinction of SMZLs into two subtypes, one with gain of 3q and the other with loss of 7q [60]. IgV_H gene analysis has revealed that some cases, in keeping with the usual

strong expression of IgM and IgD in SMZL, reveal unmutated genes, thus suggesting a naïve B-cell derivation [71, 72]. In other cases, however, a significant number of somatic mutations is detected as usually found in post-GC B-cells [71–74]. In one instance, the switch from unmutated to hypermutated elements was recorded within the same clone [75]: this suggests that the tissue microenvironment can influence the tumour and raises the question of a role for antigen in driving tumour growth. The latter point is strengthened by the observation that the use of VH gene segment in mutated cases seems to be non-random, the repetitive usage of V1-2, V1-69 and V3-34 having been recorded [71, 72]. Interestingly, as in some NMZLs, the preferential use of VH gene segment(s) might correlate with HCV infection, a fact which can explain possible tumour regression following HCV eradication [69].

Future perspectives

According to the above-mentioned data, the three types of MZL quoted in the WHO classification [6] display significant differences among them as well as certain heterogeneity within each category. There is a cogent need for a more refined tool to revise this group of lymphoid neoplasms and produce a more rationale grouping. The recent identification of the new IRTA gene family corresponding to Ig-like receptors differentially expressed in B-cells might contribute to the better understanding of MZLs [76, 77]. In particular, IRTA1 is characteristic of MZ B-cells, IRTA2 and IRTA3 are found in the GC light zone and interepithelial and interfollicular regions and IRTA4 and IRTA5 are predominantly expressed in the mantle zone [76]. Molecular studies based on single-cell or micro-dissection PCR as well as on the availability of specific monoclonal antibodies, which is reasonably expected (B. Falini, personal communication), might clarify which tumours merit the histogenetic label ‘marginal zone lymphoma’ and which do not. In the meantime, the controversies on the nodal and splenic forms of MZL should be considered to be far from their definitive solution.

Acknowledgements

This paper was supported by grants from AIRC (Milan), MIUR (Rome), Fondazione del Monte di Bologna e Ravenna, FIS Ministerio de Salud–RTIC Cáncer C10/03 (Madrid), Departamento de Educación y Cultura del Gobierno de Navarra (Pamplona), PIUNA (Pamplona) and Ruzic Research Foundation (Beverly Shores, IN).

References

- Harris N, Jaffe E, Stein H et al. A Revised European-American Classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361–1392.
- Gérard-Marchant RHI, Lennert K, Rilke F et al. Classification of non-Hodgkin’s lymphomas. *Lancet* 1974; i: 406–408.
- Stansfeld AG, Diebold J, Noel H et al. Updated Kiel classification for lymphomas. *Lancet* 1988; 1: 292–293.
- National Cancer Institute sponsored study of classifications of non-Hodgkin’s lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin’s Lymphoma Pathologic Classification Project. *Cancer* 1982; 49: 2112–2135.
- A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin’s lymphoma. The Non-Hodgkin’s Lymphoma Classification Project. *Blood* 1997; 89: 3909–3918.
- Jaffe ESHN, Stein H, Vardiman JW. Tumours of Haematopoietic and Lymphoid Tissues. International Agency for Research on Cancer 2001; 12–13.
- Longo DL. The REAL Classification of lymphoid neoplasms: one clinician’s view. In: PPO Updates. Philadelphia, PA: Lippincott 1995; 9: 1–12.
- Maes B, De Wolf-Peeters C. Marginal zone cell lymphoma—an update on recent advances. *Histopathology* 2002; 40: 117–126.
- Kurtin PJ. Marginal zone B cells, monocytoid B cells, and the follicular microenvironment. Determinants of morphologic features in a subset of low-grade B-cell lymphomas. *Am J Clin Pathol* 2000; 114: 505–508.
- Stein H, Lennert K, Mason DY et al. Immature sinus histiocytes. Their identification as a novel B-cell population. *Am J Pathol* 1984; 117: 44–52.
- Miettinen M. Histological differential diagnosis between lymph node toxoplasmosis and other benign lymph node hyperplasias. *Histopathology* 1981; 5: 205–216.
- Stein K, Hummel M, Korbjuhn P et al. Monocytoid B cells are distinct from splenic marginal zone cells and commonly derive from unmutated naïve B cells and less frequently from postgerminal center B cells by polyclonal transformation. *Blood* 1999; 94: 2800–2808.
- Isaacson PG, Spencer J. Malignant lymphoma of mucosa-associated lymphoid tissue. *Histopathology* 1987; 11: 445–462.
- Pileri S, Piccaluga PP, De Vivo A et al. Malignant lymphomas of the gastro-intestinal tract: a reappraisal on the basis of the newly proposed Revised European American Lymphoma Classification. *Ital J Gastroenterol* 1994; 26: 405–418.
- Zucca E, Roggero E, Pileri S. B-cell lymphoma of MALT type: a review with special emphasis on diagnostic and management problems of low-grade gastric tumours. *Br J Haematol* 1998; 100: 3–14.
- Isaacson PG. Gastric MALT lymphoma: from concept to cure. *Ann Oncol* 1999; 10: 637–645.
- Zucca E, Bertoni F, Roggero E, Cavalli F. The gastric marginal zone B-cell lymphoma of MALT type. *Blood* 2000; 96: 410–419.
- Cavalli F, Isaacson PG, Gascoyne RD, Zucca E. MALT Lymphomas. *Hematology (Am Soc Hematol Educ Program)* 2001: 241–258.
- Wotherspoon AC, Dogliani C, Diss TC et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993; 342: 575–577.
- Neubauer A, Thiede C, Morgner A et al. Cure of *Helicobacter pylori* infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *J Natl Cancer Inst* 1997; 89: 1350–1355.
- Bayerdorffer E, Neubauer A, Rudolph B et al. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. MALT Lymphoma Study Group. *Lancet* 1995; 345: 1591–1594.
- Caletti G, Togliani T, Fusaroli P et al. Consecutive regression of concurrent laryngeal and gastric MALT lymphoma after anti-*Helicobacter pylori* therapy. *Gastroenterology* 2003; 124: 537–543.
- Caletti G, Zinzani PL, Fusaroli P et al. The importance of endoscopic ultrasonography in the management of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *Aliment Pharmacol Ther* 2002; 16: 1715–1722.
- Du MQ. Molecular biology of gastric MALT lymphoma: application in clinical management. *Hematology* 2002; 7: 339–344.

25. Ye H, Dogan A, Karran L et al. BCL10 expression in normal and neoplastic lymphoid tissue. Nuclear localization in MALT lymphoma. *Am J Pathol* 2000; 157: 1147–1154.
26. Ohshima K, Muta H, Kawasaki C et al. Bcl10 expression, rearrangement and mutation in MALT lymphoma: correlation with expression of nuclear factor-kappaB. *Int J Oncol* 2001; 19: 283–289.
27. Chen YW, Wong KY, Au WY et al. BCL10 somatic mutations rarely occur in gastric lymphoma: detection of high frequency of polymorphisms in BCL10 coding region. *Cancer Genet Cytogenet* 2001; 127: 184–187.
28. Maes B, Demunter A, Peeters B, De Wolf-Peeters C. BCL10 mutation does not represent an important pathogenic mechanism in gastric MALT-type lymphoma, and the presence of the API2-MLT fusion is associated with aberrant nuclear BCL10 expression. *Blood* 2002; 99: 1398–1404.
29. Baens M, Maes B, Steyels A et al. The product of the t(11;18), an API2-MLT fusion, marks nearly half of gastric MALT type lymphomas without large cell proliferation. *Am J Pathol* 2000; 156: 1433–1439.
30. Liu H, Ruskon-Formestruux A, Lavergne-Slove A et al. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. *Lancet* 2001; 357: 39–40.
31. Liu H, Ye H, Dogan A et al. T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. *Blood* 2001; 98: 1182–1187.
32. Inagaki H, Okabe M, Seto M et al. API2-MALT1 fusion transcripts involved in mucosa-associated lymphoid tissue lymphoma: multiplex RT-PCR detection using formalin-fixed paraffin-embedded specimens. *Am J Pathol* 2001; 158: 699–706.
33. Lucas PC, Yonezumi M, Inohara N et al. Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J Biol Chem* 2001; 276: 19012–19019.
34. Yonezumi M, Suzuki R, Suzuki H et al. Detection of API2-MALT1 chimeric gene in extranodal and nodal marginal zone B-cell lymphoma by reverse transcription polymerase chain reaction (PCR) and genomic long and accurate PCR analyses. *Br J Haematol* 2001; 115: 588–594.
35. Starostik P, Patzner J, Greiner A et al. Gastric marginal zone B-cell lymphomas of MALT type develop along 2 distinct pathogenetic pathways. *Blood* 2002; 99: 3–9.
36. Remstein ED, Kurtin PJ, James CD et al. Mucosa-associated lymphoid tissue lymphomas with t(11;18)(q21;q21) and mucosa-associated lymphoid tissue lymphomas with aneuploidy develop along different pathogenetic pathways. *Am J Pathol* 2002; 161: 63–71.
37. Liu H, Ye H, Ruskon-Formestruux A et al. T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. *Gastroenterology* 2002; 122: 1286–1294.
38. Ye H, Liu H, Raderer M et al. High incidence of t(11;18)(q21;q21) in *Helicobacter pylori*-negative gastric MALT lymphoma. *Blood* 2003; 101: 2547–2550.
39. Okabe M, Inagaki H, Ohshima K et al. API2-MALT1 fusion defines a distinctive clinicopathologic subtype in pulmonary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. *Am J Pathol* 2003; 162: 1113–1122.
40. Streubel B, Lamprecht A, Dierlamm J et al. T(14;18)(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. *Blood* 2003; 101: 2335–2339.
41. Sanchez-Izquierdo D, Buchonet G, Siebert R et al. MALT1 is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin's lymphoma. *Blood* 2003; 101: 4539–4546.
42. Tzankov A, Hittmair A, Muller-Hermelink HK et al. Primary gastric follicular lymphoma with parafollicular monocytoid B-cells and lymphoepithelial lesions, mimicking extranodal marginal zone lymphoma of MALT. *Virchows Arch* 2002; 441: 614–617.
43. Schmid U, Cogliatti SB, Diss TC, Isaacson PG. Monocytoid/marginal zone B-cell differentiation in follicle centre cell lymphoma. *Histopathology* 1996; 29: 201–208.
44. Piris MA, Mollejo M, Campo E et al. A marginal zone pattern may be found in different varieties of non-Hodgkin's lymphoma: the morphology and immunohistology of splenic involvement by B-cell lymphomas simulating splenic marginal zone lymphoma. *Histopathology* 1998; 33: 230–239.
45. Yegappan S, Schnitzer B, Hsi ED. Follicular lymphoma with marginal zone differentiation: microdissection demonstrates the t(14;18) in both the follicular and marginal zone components. *Mod Pathol* 2001; 14: 191–196.
46. Anagnostopoulos I, Foss HD, Hummel M et al. Extranodal mantle cell lymphoma mimicking marginal zone cell lymphoma. *Histopathology* 2001; 39: 561–565.
47. Liu YX, Yoshino T, Ohara N et al. Loss of expression of alpha4beta7 integrin and L-selectin is associated with high-grade progression of low-grade MALT lymphoma. *Mod Pathol* 2001; 14: 798–805.
48. Savio A, Franzin G, Wotherspoon AC et al. Diagnosis and posttreatment follow-up of *Helicobacter pylori*-positive gastric lymphoma of mucosa-associated lymphoid tissue: histology, polymerase chain reaction, or both? *Blood* 1996; 87: 1255–1260.
49. Thiede C, Wundisch T, Alpen B et al. Long-term persistence of monoclonal B cells after cure of *Helicobacter pylori* infection and complete histologic remission in gastric mucosa-associated lymphoid tissue B-cell lymphoma. *J Clin Oncol* 2001; 19: 1600–1609.
50. Montalban C, Santon A, Boixeda D et al. Treatment of low grade gastric mucosa-associated lymphoid tissue lymphoma in stage I with *Helicobacter pylori* eradication. Long-term results after sequential histologic and molecular follow-up. *Haematologica* 2001; 86: 609–617.
51. Alpen B, Thiede C, Wundisch T et al. Molecular diagnostics in low-grade gastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type after *Helicobacter pylori* eradication therapy. *Clin Lymphoma* 2001; 2: 103–108.
52. Bertoni F, Conconi A, Capella C et al. Molecular follow-up in gastric mucosa-associated lymphoid tissue lymphomas: early analysis of the LY03 cooperative trial. *Blood* 2002; 99: 2541–2544.
53. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; 338: 1175–1176.
54. Nathwani BN, Anderson JR, Armitage JO et al. Marginal zone B-cell lymphoma: a clinical comparison of nodal and mucosa-associated lymphoid tissue types. Non-Hodgkin's Lymphoma Classification Project. *J Clin Oncol* 1999; 17: 2486–2492.
55. Nathwani BN, Drachenberg MR, Hernandez AM et al. Nodal monocytoid B-cell lymphoma (nodal marginal-zone B-cell lymphoma). *Semin Hematol* 1999; 36: 128–138.
56. Berger F, Felman P, Thieblemont C et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. *Blood* 2000; 95: 1950–1956.
57. Conconi A, Bertoni F, Pedrinis E et al. Nodal marginal zone B-cell lymphomas may arise from different subsets of marginal zone B lymphocytes. *Blood* 2001; 98: 781–786.
58. Marasca R, Vaccari P, Luppi M et al. Immunoglobulin gene mutations and frequent use of VH1-69 and VH4-34 segments in hepatitis C virus-positive and hepatitis C virus-negative nodal marginal zone B-cell lymphoma. *Am J Pathol* 2001; 159: 253–261.
59. Ott MM, Rosenwald A, Katzenberger T et al. Marginal zone B-cell lymphomas (MZBL) arising at different sites represent different biological entities. *Genes Chromosom Cancer* 2000; 28: 380–386.

60. Solé F, Salido M, Espinet B et al. Splenic marginal zone B-cell lymphomas: two cytogenetic subtypes, one with gain of 3q and the other with loss of 7q. *Haematologica* 2001; 86: 71–77.
61. Melo JV, Robinson DS, Gregory C, Catovsky D. Splenic B cell lymphoma with ‘villous’ lymphocytes in the peripheral blood: a disorder distinct from hairy cell leukemia. *Leukemia* 1987; 1: 294–298.
62. Kent SA, Variakojis D, Peterson LC. Comparative study of marginal zone lymphoma involving bone marrow. *Am J Clin Pathol* 2002; 117: 698–708.
63. Franco V, Florena AM, Iannitto E. Splenic marginal zone lymphoma. *Blood* 2003; 101: 2464–2472.
64. Franco V, Florena AM, Stella M et al. Splenectomy influences bone marrow infiltration in patients with splenic marginal zone cell lymphoma with or without villous lymphocytes. *Cancer* 2001; 91: 294–301.
65. Thieblemont C, Felman P, Berger F et al. Treatment of splenic marginal zone B-cell lymphoma: an analysis of 81 patients. *Clin Lymphoma* 2002; 3: 41–47.
66. Chacon JI, Mollejo M, Munoz E et al. Splenic marginal zone lymphoma: clinical characteristics and prognostic factors in a series of 60 patients. *Blood* 2002; 100: 1648–1654.
67. Thieblemont C, Felman P, Callet-Bauchu E et al. Splenic marginal-zone lymphoma: a distinct clinical and pathological entity. *Lancet Oncol* 2003; 4: 95–103.
68. Parry-Jones N, Matutes E, Gruszka-Westwood AM et al. Prognostic features of splenic lymphoma with villous lymphocytes: a report on 129 patients. *Br J Haematol* 2003; 120: 759–764.
69. Arcaini L, Paulli M, Boveri E et al. Marginal zone-related neoplasms of splenic and nodal origin. *Haematologica* 2003; 88: 80–93.
70. Camacho FI, Mollejo M, Mateo MS et al. Progression to large B-cell lymphoma in splenic marginal zone lymphoma: a description of a series of 12 cases. *Am J Surg Pathol* 2001; 25: 1268–1276.
71. Algara P, Mateo MS, Sanchez-Beato M et al. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. *Blood* 2002; 99: 1299–1304.
72. Bahler DW, Pindzola JA, Swerdlow SH. Splenic marginal zone lymphomas appear to originate from different B cell types. *Am J Pathol* 2002; 161: 81–88.
73. Dunn-Walters DK, Boursier L, Spencer J, Isaacson PG. Analysis of immunoglobulin genes in splenic marginal zone lymphoma suggests ongoing mutation. *Hum Pathol* 1998; 29: 585–593.
74. Miranda RN, Cousar JB, Hammer RD et al. Somatic mutation analysis of IgH variable regions reveals that tumor cells of most parafollicular (monocytoid) B-cell lymphoma, splenic marginal zone B-cell lymphoma, and some hairy cell leukemia are composed of memory B lymphocytes. *Hum Pathol* 1999; 30: 306–312.
75. Zhu D, Orchard J, Oscier DG et al. V(H) gene analysis of splenic marginal zone lymphomas reveals diversity in mutational status and initiation of somatic mutation *in vivo*. *Blood* 2002; 100: 2659–2661.
76. Hatzivassiliou G, Miller I, Takizawa J et al. IRTA1 and IRTA2, novel immunoglobulin superfamily receptors expressed in B cells and involved in chromosome 1q21 abnormalities in B cell malignancy. *Immunity* 2001; 14: 277–289.
77. Miller I, Hatzivassiliou G, Cattoretti G et al. IRTAs: a new family of immunoglobulinlike receptors differentially expressed in B cells. *Blood* 2002; 99: 2662–2669.

Note added in proof

After the acceptance of the present paper, Falini et al. published on the first application of anti-IRTA1 specific antibodies to the analysis of normal lymphoid tissues (Falini B, Tiacci E, Pucciarini A et al. Expression of the IRTA1 receptor identifies intraepithelial and subepithelial marginal zone B cells of the mucosa-associated lymphoid tissue (MALT). *Blood* 2003; 102: 3684–3692). IRTA1 was selectively expressed by a B-cell population located underneath and within the tonsil epithelium and dome epithelium of Peyer patches. In contrast, no or a low number of IRTA1+ cells was usually observed in the MZ of mesenteric lymph nodes and spleen. Interestingly, monocytoid B cells in reactive lymph nodes were strongly positive. Further studies on MZLs are ongoing at present with the same antibodies.