

## Biclonality of Gastric Lymphomas

Antonello D. Cabras, Sonja Candidus, Falko Fend, Marcus Kremer, Stephan Schulz, Cesare Bordi, Gregor Weirich, Heinz Höfler, and Martin Werner

*Departments of Pathology (SC, FF, MK, SS, GW, HH, MW), Technische Universität München, München, Germany; and GSF-National Research Center for Environment and Health, Neuherberg (ADC, HH), Germany, and Parma (CB), Italy*

**SUMMARY:** The pathogenesis and clonal evolution of gastric diffuse large B-cell lymphoma (DLBCL) and its relationship to extranodal marginal zone B-cell lymphoma (MZBL), mucosa-associated lymphoid tissue (MALT) type, are still controversial. The aim of this study was to establish the clonality of morphologically distinct areas of gastric lymphomas as well as their genetic relationship to each other. Six gastric lymphomas, consisting of two MZBL, MALT type, two DLBCL, and two "composite" lymphomas were subjected to laser capture microdissection and subsequent PCR-based amplification of the immunoglobulin heavy chain gene. One DLBCL showed a biclonal pattern of rearranged immunoglobulin heavy chain (IgH) genes of two different areas without evidence of a common origin. Two composite DLBCL with areas of extranodal MZBL, MALT type, were also biclonal and displayed different IgH gene rearrangements in the small-cell and in the large-cell components, respectively. Sequencing of the CDR3 region revealed unique VH-N-D and D-N-JH junctions, thus corroborating the presence of two genuinely distinct tumor clones in each of these three cases. In contrast, the remaining three gastric lymphomas (one DLBCL and two MZBL, MALT type) showed IgH gene rearrangements in which CDR3 regions were identical in the different tumor areas. Our results suggest that gastric DLBCL may be composed of more than one tumor cell clone. Further, DLBCL may not necessarily evolve by transformation of a low-grade lymphoma, but may also originate *de novo*. An ongoing emergence of new tumor clones may considerably hamper molecular diagnosis and follow-up of gastric DLBCL. (*Lab Invest* 2001, 81:961-967).

The two main groups of gastric lymphoma are extranodal marginal zone B-cell lymphoma (MZBL), mucosa-associated lymphoid tissue (MALT) type, and diffuse large B-cell lymphoma (DLBCL) (Jaffe et al, 1999). MZBL, MALT type, is composed of a monotonous, diffuse infiltrate of small lymphoid cells. DLBCL is characterized by a diffuse infiltrate of large malignant lymphoid blasts resembling centroblasts, plasmablasts, and immunoblasts. Occasionally, DLBCL shows both small- and large-cell components. These tumors have been called "composite" lymphomas, their histologic spectrum ranging from cases with well separated small- and large-cell components, to tumors in which these components are intermingled (De Jong et al, 1997; De Wolf-Peters and Achten, 1999).

The histogenesis of gastric DLBC lymphoma, as well as its possible clonal relationship to concomitant or preceding low-grade components, has been strongly debated recently and is still not fully clarified. The clonality of B-cell non-Hodgkin's lymphomas can be assessed by the PCR analysis of rearranged immunoglobulin heavy chain (IgH) genes that vary in size in different neoplastic cell populations (Trainor et al, 1991). Several studies comparing the patterns of clonal IgH gene rearrangement between large-cell and

small-cell areas in the same gastric B-cell lymphoma have demonstrated a common clonal origin of both components (Chan et al, 1990; Du et al, 1996a, 1996b; Peng et al, 1997). These results suggest that the DLBCL may have developed by transformation of the MZBL, MALT type, component (De Wolf-Peters and Tierens, 1998). In contrast, Matolcsy et al (1999) found no clonal relationship between well separated small-cell and large-cell components of one case of a multifocal gastric lymphoma.

The aim of the present study was to establish the clonality in different areas of two gastric MZBL, MALT type, two gastric DLBCL, and two composite gastric DLBCL with areas of MZBL, MALT type, as well as their genetic relationships with one another.

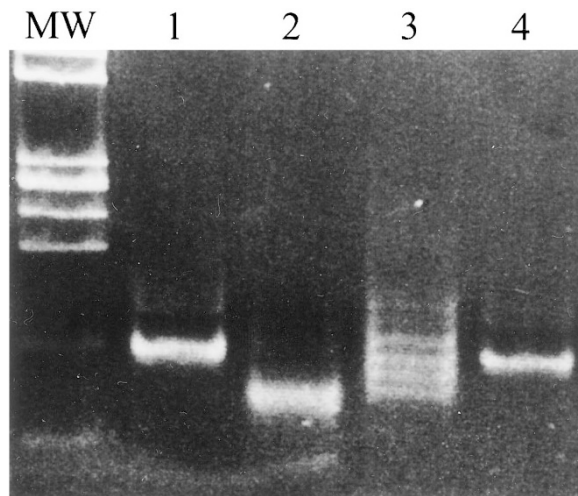
### Results

#### PCR, Agarose Gel Analysis

PCR amplification of the IgH genes in the different microdissected tumor samples yielded sharp bands of the same sizes in the two MZBL, MALT type (Cases 1 and 2), as well as in the two DLBCL (Cases 3 and 4). PCR amplification yielded bands of varying sizes in the two DLBCL with areas of MZBL, MALT type only (Cases 5 and 6). A representative example is shown in Figure 1 (Case 5). The dominant clones of each tumor area were found in PCR of both the microdissected tissues and the entire sections. In Cases 5 and 6, however, PCR-based DNA amplification of the entire sections only revealed the clone of the large-cell component. PCR amplification of DNA derived from

Received January 29, 2001.

Address reprint requests to: Dr. Antonello D. Cabras, Institute of Pathology, GSF-National Research Center for Environment and Health, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany. E-mail: ad.cabras@lrz.tum.de



**Figure 1.**

Ethidium bromide-stained 3% agarose gel of Frame Region 3, PCR products from Case 5. Lane MW, molecular-weight marker; Lane 1, diffuse large B-cell lymphoma (DLBCL) component; Lane 2, marginal zone B-cell lymphoma (MZBL) component; Lane 3, polyclonal control; Lane 4, monoclonal control.

reactive hyperplastic lymphoid tissue (chronic tonsillitis) revealed a polyclonal pattern with and without microdissection (data not shown). PCR amplicons ranged from 83 to 128 bp. PCR products from different tumor areas were identical in size (92 bp in Case 1, 128 bp in Case 2, 98 bp in Cases 3 and 4, respectively) in the two MZBL, MALT type (Cases 1 and 2), and the two DLBCL (Cases 3 and 4). PCR products from well-separated components differed in size in one composite lymphoma (Case 5; 119 bp in the DLBCL and 95 bp in the MZBL, MALT type). PCR products of distinct sizes were also identified in a second composite lymphoma with intermingling MZBL, MALT type, and DLBCL components (95 bp in the MZBL, MALT type, component and 83 bp in the DLBCL component).

### Sequence Analysis

All CDR3 sequences were *in frame*. The nucleotide and deduced amino acid sequences are shown in Figure 2, A and B. D- and JH-genes were detected in all six cases (11 specimens). Four D-genes matched database sequences (germline), whereas another seven showed single nucleotide deviations from database entries (20% and less). Fusions of two D-regions were found in two cases (3 and 5). No preferential utilization of D-genes was apparent, whereas JH4 segments were identified in five cases (seven specimens). In six specimens, germline JH genes were found, whereas sequence deviations ranging from 75% to 96% were apparent in three specimens.

In both MZBL, MALT type, as well as in one DLBCL, identical sequences of the D- and JH segments were found in specimens from antrum and corpus mucosa (Cases 1, 2, and 3, Fig. 2A). In the second DLBCL, however, tumor infiltrates of antrum and corpus mucosa displayed different sequences of VH-(N)-D and D-(N)-JH junctions, solely present in each compart-

ment (Case 4, Fig. 2B). Also, small- and large-cell components of the two composite lymphomas showed individual sequences of VH-(N)-D and D-(N)-JH junctions (Cases 5 and 6, Fig. 2B).

### Intraclonal Variations

In one composite lymphoma (Case 6), multiple sequence variations were identified among different PCR clones derived from the small-cell component: two replacement (R) and two silent (S) mutations were observed in the D-region. One R- and three S-mutations, and a deletion of three nucleotides were observed in the joining region (Fig. 2B).

### Discussion

Gastric DLBCL are thought to develop either as a clonal progression from a preexisting MZBL, MALT type, tumor or independently from the coexisting small-cell components, ie *de novo* (De Wolf-Peeters and Achten, 1999; Isaacson 1996). Evidence for both hypotheses has been drawn from the detection of either identical (Chan et al, 1990; Du et al 1996a, 1996b) or distinct (Matolcsy et al, 1999) B-cell clones in composite DLBCL with coexisting MZBL, MALT type. Whereas these previous studies aimed at the genetic relationship between small- and large-cell components in relatively rare composite lymphomas, the question of whether gastric DLBCL without a concomitant small-cell component are monoclonal or oligoclonal malignancies has not yet been addressed.

In our study, three gastric lymphomas (two composite lymphomas and one DLBCL) exhibited biclonal IgH genes. The fact that both unique sequences in each of these tumors were *in frame* indicates the presence of two different tumor clones in each lymphoma. Based on the isolation of pure tumor cell populations by laser capture microdissection (LCM), these sequences were truly derived from tumor cells alone. One composite lymphoma (Case 5) with well-separated large-cell and small-cell areas, exhibited distinct CDR3 regions in both, indicating that neither cell type had evolved from the other. Immunohistochemical demonstration of Ig light chain restriction in the small-cell but not the large-cell component further corroborated the genetic findings. A similar situation was found in the second composite lymphoma (Case 6), in which, in contrast to the above-mentioned case, large and small tumor cells were intermingled. After being separated by LCM, these cell types also displayed distinct CDR3 regions. Therefore, the DLBCL components in these two composite lymphomas seem not to have arisen from transformed cells of the adjacent MZBL, MALT type, but rather independently. It is therefore not unlikely that DLBCL in composite lymphomas originates *de novo* rather than evolving by transformation of a low-grade lymphoma. However, theoretically, these DLBCL components may also have arisen from transformed low-grade progenitors no longer present in the tumor. Histologically, one of the three biclonal tumors, a DLBCL, showed phenotypically identical



lymphoid blasts at all sites. In this case, the microdissected tumor cells from antrum and corpus mucosa showed different VH-N-D and D-N-JH junctions as evidence of distinct tumor clones.

Biclonality in different cell compartments of the same tumor or at different anatomical sites may be evidence of ongoing antigenic stimulation. In all tumor samples except for both composite lymphomas, we were able to detect the same clonal products from entire sections as well as from the microdissected tissues. These results suggest that the amplicates from the microdissected samples were not derived from minor clonal populations of uncertain biologic significance, or PCR artifacts, but in fact represent the malignant cells. In the two composite lymphomas, only one of the two clones obtained from microdissected tissue was detectable in PCR from whole sections. This fact probably reflects preferential amplification, either due to different amplification efficiencies based on differences in the primer binding sites or to different sizes of the malignant clones.

In our study, DNA was obtained by LCM, a technique that was not used in previous studies on gastric lymphomas. This may explain why most previous studies found evidence for the presence of only a single tumor cell population in gastric lymphomas (Aiello et al, 1999; Nakamura et al, 1998; Peng et al, 1997). Also, interpretation of PCR products of differing sizes as being polyclonal (Torlakovic et al, 1997) may explain why oligoclonality has not been detected more frequently in gastric lymphomas.

Biclonality of non-Hodgkin's lymphomas with unrelated tumor clones arising at the same anatomical site has already been demonstrated in a few nodal and extranodal lymphomas by PCR IgH gene analyses. Biclonality of the rare nodal or extranodal composite lymphomas has been reported previously and it was suggested that different clones within such tumors originate from different progenitor cells (Fend et al, 1999). DLBCL arising in the setting of a concomitant small-cell lymphoma has also occasionally been found as distinct clonal tumor cell populations in both nodal and extranodal sites (Matolcsy et al, 1995, 1999). Biclonality in phenotypically identical tumor areas at the same anatomic site as found in one of our gastric DLBCL, however, has only rarely been demonstrated before. Sklar et al (1984) detected biclonal follicular, small cleaved-cell lymphomas in single lymph nodes from two patients. In another study, one case of a gastric MZBL, MALT type, was found to harbor two tumor clones with differently rearranged CDR3 regions (Zucca et al, 1998). Another case of MZBL, MALT type, of the salivary gland showed genuinely different clones with distinct CDR3 regions and VH and DH genes in consecutive biopsy specimens (Lasota and Miettinen, 1997). The coexistence of phenotypically identical tumor clones with different rearranged IgH genes in low-grade B-cell tumors are in accordance with our findings of more than one neoplastic tumor clone in gastric DLBCL.

The presence of biclonality in three gastric lymphomas (Cases 4 to 6) is especially interesting in the

context of *Helicobacter pylori* (HP) infection, which was detected in these cases. On the other hand, the three monoclonal gastric lymphomas were negative for HP. These findings support an HP-linked pathogenesis of gastric lymphomas (Wotherspoon et al, 1991). Further evidence of this pathway stems from the finding of single nucleotide variations in the CDR3 region of one composite lymphoma (Case 6). Similar variations have previously been interpreted as indicators of a postgerminal center origin (Du et al 1996a, 1996b; Hallas et al, 1998). An autoimmune response, chiefly triggered by HP-associated immune phenomena, may also play an important role in the development of gastric lymphomas (Greiner et al, 1994; Hussell et al, 1996). Chronic HP-gastritis may lead to non-neoplastic B-cell proliferation in the mucosa (Nakamura et al, 1998), the neoplastic transformation of which is dependent on mutations in tumor suppressor genes or oncogenes (Neumeister et al 1997; Peng et al 1996). Ott et al (1994) showed that inflammatory lymphoid infiltrates outside the tumor mass displayed additional clonal B-cell populations unrelated to the primary tumor in two cases of gastric MZBL, MALT type. These authors hypothesized that additional clonal cell populations may develop apart from the primary tumor caused by continued triggering by the autoimmune response. Our biclonal gastric lymphomas may have developed from different non-neoplastic clonal cell populations in HP-positive gastritis leading to unrelated tumor clones.

Biclonality of gastric DLBCL may hamper the molecular genetic analysis of these tumors. PCR analysis of the IgH gene for the detection of minimal residual disease or relapse after therapy may reveal clones differing from those found in the primary tumor. Finally, in biclonal tumor cell populations, the molecular biological tumor characteristics, such as specific gene mutations or the pattern of gene expression associated with tumor prognosis or therapeutic response, may be rather heterogeneous.

## Materials and Methods

### Material and Clinical Data

Ten formalin-fixed, paraffin-embedded tissue blocks from six gastric lymphomas were retrieved from the files of the Department of Pathology, University of Parma. The material was derived from gastrectomy specimens. The histologic classification of lymphomas was carried out in accordance with defined histopathologic criteria (Jaffe et al, 1999) using 5- $\mu$ m sections stained with hematoxylin and eosin and Giemsa. Specimens consisted of two MZBL, MALT type, two DLBCL, and two composite DLBCL with areas of MZBL, MALT type, lymphoma. Of the two composite lymphomas, one case (Case 5) had well separated small-cell and large-cell components, whereas in the other case (Case 6) these cell types were intermingled. Tumor blocks from antrum and corpus mucosa were analyzed in Cases 1 to 4, and from antrum mucosa in Cases 5 and 6. The tumor cells of the six cases



examined were immunohistochemically positive for CD20 and CD79a, and negative for CD3, CD5, CD10, CD23, IgA, IgG, IgD, IgM, and  $\lambda$ -chain. Light chain restriction ( $\kappa$ ) was found in one DLBC lymphoma (Case 4) and in the small-cell component ( $\kappa$ ) of a composite lymphoma (Case 5). Three cases were HP-positive by immunohistochemistry. The Musshoff modification of the Ann Arbor system was used for tumor staging (Musshoff, 1977). Table 1 summarizes the histopathologic and clinical data.

### Preparation of DNA

The tumor cells ( $3 \times 10^3$  per sample) were isolated under microscopic control by LCM from 10  $\mu$ m tissue sections previously stained with hematoxylin and eosin for 15 seconds. LCM was performed using a PixCell laser capture microscope (Arcturus Engineering, Santa Clara, California) as described previously (Fend et al, 1999) (Fig. 3). In the DLBCL and MZBL, MALT type, the tumor cells were isolated from two different tumor blocks, ie corpus and antrum (two samples per case), and in the composite lymphomas from DLBCL and MZBL, MALT type, components of one tumor block (two samples per case), respectively. To investigate whether the dominant clone(s) found in the microdissected samples were also detectable in whole tissue sections, DNA was isolated from consecutive sections of all 10 tissue blocks without microdissection. DNA-quality was assessed by amplification of the  $\beta$ -actin pseudogene (Ghossein et al, 1994; Moos and Gallwitz, 1983). For all PCR experiments, a metal block thermal cycler was used (PTC-200; MJ Research, Watertown, Massachusetts).

### IgH Gene Rearrangements

DNA amplification for the IgH gene rearrangements was carried out as a single-step PCR according to a published protocol (Segal et al, 1994). Primer sequences were FR3: 5'-ACA CGG C(C/T)(G/C) TGT ATT ACT GT-3' and LJH: 5'-TGA GGA GAC GGT GAC C-3'. A monoclonal positive control (isolated from paraffin sections of a case of nodal DLBCL) and a negative control ( $H_2O$  instead of DNA) were amplified

with the lymphoma samples. As a polyclonal control, DNA isolated from paraffin sections of a case of chronic tonsillitis was used. For all tumor and control samples, the PCR analyses from the DNA samples obtained with and without tissue microdissection were performed in three independent experiments.

### Agarose Gel Electrophoresis

The PCR amplicates were electrophoresed on 2.5% agarose gels (FMC BioProducts, Rockland, Maine) for the  $\beta$ -actin pseudogene, and on 3% intermediate-melting agarose gels (Metaphor; FMC BioProducts) for the IgH gene rearrangements, followed by DNA visualization with ethidium bromide.

### Sequencing of the CDR3 Region

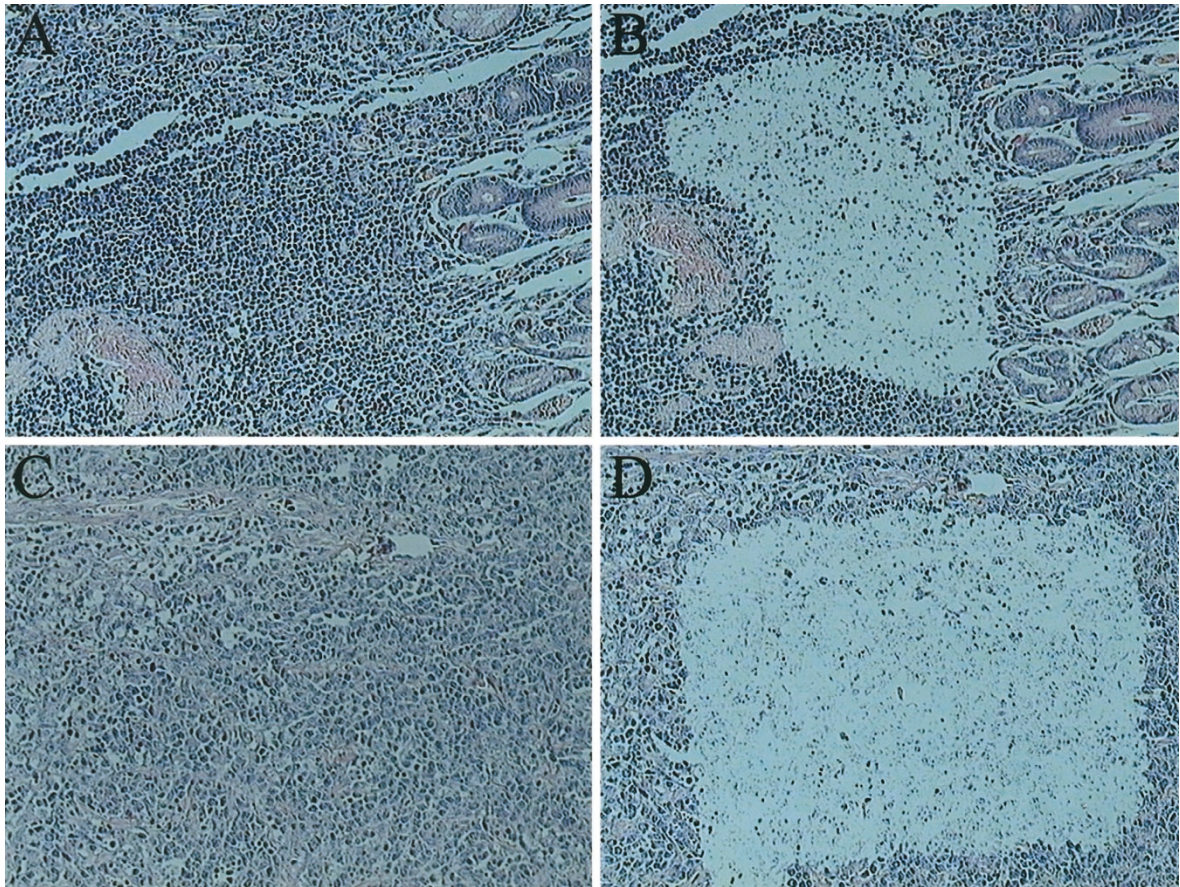
For purification, PCR-products were electrophoresed on low-melting agarose gels (NuSieve GTG agarose 1.5%; FMC BioProducts). Twelve microdissected samples from six patients yielded sharp clonal bands that were excised and amplicons recovered using QIAquick Gel extraction kit (Qiagen GmbH, Hilden, Germany). The purified PCR products were further processed with *Pfu* DNA polymerase for blunt ends, ligated into pPCR-Script amp SK(+) cloning vector, and then used for transformation of *E. coli* according to the manufacturer's protocol (XL10-Gold Kan ultra-competent cells, PCR-Script amp cloning kit; Stratagene Cloning Systems, La Jolla, California). For each PCR fragment, 10 clones were selected and sequenced. The plasmids were purified using QIAprep Miniprep Kit (Qiagen) and cycle sequenced using a plasmid specific forward primer (T3; Stratagene). Cycle sequencing was performed using BIG dye-deoxy terminators (Applied Biosystems, Foster City, California). Sequencing reactions were separated on 10% polyacrylamide gels (Long Ranger; FMC BioProducts) in an automated sequencer (ABI 377; Applied Biosystems).

The sequences of the expressed D- and JH-segments were compared with those of the published germline D-, DIR-, and JH-segments using the DNASIS v.2.6 program and the IMGT database (Lefranc

**Table 1. Clinicopathological Data of Cases Analyzed**

Classification	Staging	M/F	Age	HP	Follow Up
MZBL					
Case 1	EI1	F	50	neg	27 months AWD
Case 2	EII1	M	70	neg	7 months ANED
DLBCL					
Case 3	EII1	M	66	neg	156 months ANED
Case 4	EI2	M	72	pos	8 months DOD
Composite					
DLBCL/MZBL					
Case 5	EI2	M	61	pos	120 months ANED
Case 6	EI2	M	59	pos	142 months ANED

M/F, male/female; HP, *Helicobacter pylori*; DOD, dead of disease; ANED, alive with no evidence of disease; AWD, alive with disease; MZBL, marginal zone B-cell lymphoma; DLBCL, diffuse large B-cell lymphoma.



**Figure 3.**

A, MZBL component of Case 5 before laser capture microdissection (LCM). B, MZBL component of Case 5 after LCM. C, DLBCL component of Case 5 before LCM. D, DLBCL component of Case 5 after LCM.

et al, 1999). The sequences were aligned using the stringent criteria defined by Corbett et al (1997).

### Acknowledgement

The authors are especially thankful to Birgit Geist for expert technical assistance.

### References

Aiello A, Giardini R, Tondini C, Balzarotti M, Diss T, Peng H, Delia D, and Pilotti S (1999). PCR-based clonality analysis: A reliable method for the diagnosis and follow-up monitoring of conservatively treated gastric B-cell MALT lymphomas? *Histopathology* 34:326–330.

Chan JK C, Ng CS, and Isaacson PG (1990). Relationship between high-grade lymphoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma (MALToma) of the stomach. *Am J Pathol* 136:1153–1164.

Corbett SJ, Tomlinson IA, Sonnhammer ELL, Buck D, and Winter G (1997). Sequence of the immunoglobulin diversity (D) segment locus: A systematic analysis provides no evidence for the use of DIR segments, inverted D segments, “minor” D segments or D-D recombination. *J Mol Biol* 270:587–597.

De Jong D, Boot H, Van Heerde P, Hart GAM, and Taal BG (1997). Histological grading in gastric lymphoma: Pretreatment criteria and clinical relevance. *Gastroenterology* 112: 1466–1474.

De Wolf-Peeters C and Achten R (1999). The histogenesis of large-cell gastric lymphomas. *Histopathology* 34:71–75.

De Wolf-Peeters C and Tierens A (1998). Controversies in MALT lymphoma classification, low and high grade. *Histopathology* 32:277–278.

Du M, Diss TC, Xu C, Peng H, Isaacson PG, and Pan L (1996a). Ongoing mutation in MALT lymphoma immunoglobulin gene suggests that antigen stimulation plays a role in the clonal expansion. *Leukemia* 10:1190–1197.

Du M, Xu C, Diss TC, Peng H, Wotherspoon AC, Isaacson PG, and Pan L (1996b). Intestinal dissemination of gastric mucosa-associated lymphoid tissue lymphoma. *Blood* 88: 4445–4451.

Fend F, Quintanilla-Martinez L, Kumar S, Beaty MW, Blum L, Sorbara L, Jaffe ES, and Raffeld M (1999). Composite low-grade B-cell lymphomas with two immunophenotypically distinct cell populations are true biclonal lymphomas: A molecular analysis using laser capture microdissection. *Am J Pathol* 154:1857–1866.

Ghossein RA, Ross DG, Salomon RN, and Rabson AR (1994). A search for mycobacterial DNA in sarcoidosis using the polymerase chain reaction. *Am J Clin Pathol* 101:733–737.



- Greiner A, Marx A, Heesemann J, Leebmann J, Schmausser B, and Müller-Hermelink HK (1994). Idiotype identity in a MALT-type lymphoma and B-cells in *Helicobacter pylori* associated chronic gastritis. *Lab Invest* 70:572–578.
- Hallas C, Greiner A, Peters K, and Müller-Hermelink HK (1998). Immunoglobulin VH genes of high-grade mucosa-associated lymphoid tissue lymphomas show a high load of somatic mutations and evidence of antigen-dependent affinity maturation. *Lab Invest* 78:277–287.
- Hussell T, Isaacson PG, Crabtree JE, and Spencer J (1996). *Helicobacter pylori*-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B-cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *J Pathol* 178:122–127.
- Isaacson PG (1996). Recent developments in our understanding of gastric lymphomas. *Am J Surg Pathol* 20:1–7.
- Jaffe ES, Harris NL, Diebold J, and Müller-Hermelink HK (1999). World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: A progress report. *Am J Clin Pathol* 111:8–12.
- Lasota J and Miettinen M (1997). Coexistence of different B-cell clones in consecutive lesions of low-grade MALT lymphoma of the salivary gland in Sjögren's disease. *Mod Pathol* 10:872–878.
- Lefranc MP, Giudicelli V, Ginestoux C, Bodmer J, Muller W, Bontrop R, Lemaitre M, Malik A, Barbie B, and Chaume D (1999). IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res* 27:209–212.
- Matolcsy A, Casali P, and Knowles DM (1995). Different clonal origin of B-cell populations of chronic lymphocytic leukemia and large-cell lymphoma in Richter's syndrome. *Ann NY Acad Sci* 764:496–503.
- Matolcsy A, Nagy M, Kisfaludy N, and Kelényi, G (1999). Distinct clonal origin of low-grade MALT-type and high-grade lesions of a multifocal lymphoma. *Histopathology* 34:6–8.
- Moos M and Gallwitz D (1983). Structure of two human beta-actin related processed genes one of which is located next to a simple repetitive sequence. *EMBO J* 2:757–761.
- Musshoff K (1977). Clinical staging classification of non-Hodgkin's lymphomas. *Strahlentherapie* 153:218–221.
- Nakamura S, Aoyagi K, Furuse M, Suekane H, Matsumoto T, Yao T, Sakai Y, Fuchigami T, Yamamoto I, Tsuneyoshi M, and Fujishima M (1998). B-cell monoclonality precedes the development of gastric MALT lymphoma in *Helicobacter pylori* associated chronic gastritis. *Am J Pathol* 152:1271–1279.
- Neumeister P, Hoefler G, Beham-Schmid C, Schmidt H, Apfelbeck U, Schaidt H, Linkesch W, and Sill H (1997). Deletion analysis of the p16 tumor suppressor gene in gastrointestinal mucosa-associated lymphoid tissue lymphomas. *Gastroenterology* 112:1871–1875.
- Ott MM, Linke B, Gerhard N, Kneba M, Greiner A, Ott G, and Müller-Hermelink (1994). Characterization of clonal B-cell populations in gastric lymphomas of MALT and related chronic gastritis using the polymerase chain reaction. *Verh Dtsch Ges Pathol* 78:302–304.
- Peng H, Chen G, Du MQ, Singh N, Isaacson PG, and Pan LX (1996). Replication error phenotype and p53 gene mutation in lymphomas of mucosa-associated lymphoid tissue. *Am J Pathol* 148:643–648.
- Peng H, Du M, Diss TC, Isaacson PG, and Pan L (1997). Genetic evidence for a clonal link between low and high-grade components in gastric MALT B-cell lymphoma. *Histopathology* 30:425–429.
- Segal GH, Jorgensen T, Masih AS, and Braylan RC (1994). Optimal primer selection for clonality assessment by polymerase chain reaction analysis: I. Low-grade B-cell lymphoproliferative disorders of nonfollicular center cell type. *Hum Pathol* 25:1269–1275.
- Sklar J, Cleary ML, Thielemans K, Gralow J, Warnke R, and Levy R (1984). Biclinal B-cell lymphoma. *N Engl J Med* 311:20–27.
- Torlakovic E, Cherwitz DL, Jessurun J, Scholes J, and McGlennen R (1997). B-cell gene rearrangement in benign and malignant lymphoid proliferations of mucosa-associated lymphoid tissue and lymph nodes. *Hum Pathol* 28:166–173.
- Trainor KJ, Brisco MJ, Wan JH, Neoh S, Grist S, and Morley AA (1991). Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. *Blood* 78:192–196.
- Wotherspoon AC, Ortis-Hidalgo C, Falzon MR, and Isaacson PG (1991). *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 338:1175–1176.
- Zucca E, Bertoni F, Roggero E, Bosshard G, Cazzaniga G, Pedrinis E, Biondi A, and Cavalli F (1998). Molecular analysis of the progression from the *Helicobacter pylori*-associated chronic gastritis to mucosa-associated lymphoid-tissue lymphoma of the stomach. *N Engl J Med* 338:804–833.