T(11;18) Is a Marker for All Stage Gastric MALT Lymphomas That Will Not Respond to *H. pylori* Eradication

HONGXIANG LIU,* HONGTAO YE,* AGNES RUSKONE–FOURMESTRAUX,[†,§] DAPHNE DE JONG,[‖] STEFANO PILERI,[¶] CHRISTIAN THIEDE,[#] ANNE LAVERGNE,[§,**] HENK BOOT,[‖] GIANCARLO CALETTI,[¶] THOMAS WÜNDISCH,[††] THIERRY MOLINA,[†,§] BABS G. TAAL,[‖] SABATTINI ELENA,[¶] TOGLIANI THOMAS,[¶] PIER LUIGI ZINZANI,[¶] ANDREAS NEUBAUER,[††] MANFRED STOLTE,[§§] RIFAT A. HAMOUDI,* AHMET DOGAN,* PETER G. ISAACSON,* and MING–QING DU*

*Department of Histopathology, University College London, London, England; [†]Service de Gastro-enterologie/Service d'Anatomie Pathologique, Hotel-Dieu, Ap-Hp, Paris, France; [§]Groupe d'Etude des Lymphomes Digestifs, Fondation Francaise de Cancérologie Digestive, France; [‖]Department of Pathology/Gastroenterology, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [¶]Servizi di Anatomia Patologica e Gastroenterologia, Università degli Studi di Bologna, Bologna, Italy; [#]Labor Molekulare Diagnostik, Universitätsklinikum Carl Gustav Carus, Dresden, Germany; **Service d'Anatomie Pathologique, Hopital Lariboisiere, Paris, France; [††]Department of Hematology, Oncology and Immunology, Philipps University Marburg, Marburg, Germany; and [§§]Institut fur Pathologie, Klinikum Bayreuth, Bayreuth, Germany

Background & Aims: **Eradication of *Helicobacter pylori* leads to cure of gastric mucosa-associated lymphoid tissue (MALT) lymphoma in 75% of localized cases. However, prolonged follow-up is necessary to determine whether a lymphoma responds to therapy. In a small series of cases, we showed that t(11;18)(q21;q21)-positive MALT lymphomas failed to respond to *H. pylori* eradication. The present study aimed to verify this finding in a large cohort and confirm whether the translocation predicts the response of stage I_E tumors, for which clinical staging has little prognostic value.** ***Methods:*** **A total of 111 patients with *H. pylori*–positive gastric MALT lymphoma treated with antibiotics were studied. Clinical staging was undertaken before therapy. The response of lymphoma to *H. pylori* eradication was determined by histologic examination of gastric biopsy specimens. Diagnostic biopsy specimens were analyzed for t(11;18)(q21;q21) by reverse-transcription polymerase chain reaction of the API2-MALT1 transcript.** ***Results:*** **Forty-seven of the 48 patients who showed complete regression had lymphoma at stage I_E, whereas 43 of the 63 nonresponsive cases were at stage I_E and the remaining cases at stage II_E or above. t(11;18)(q21;q21) was detected in 2 of 48 complete-regression cases, and these positive cases showed relapse of lymphoma in the absence of *H. pylori* reinfection. In contrast, the translocation was present in 42 of the 63 nonresponsive cases, including 26 of 43 (60%) at stage I_E.** ***Conclusions:*** **t(11;18)(q21;q21)-positive gastric MALT lymphomas, including those at stage I_E, do not respond to *H. pylori* eradication. Detection of the translocation should help the clinical management of patients with gastric MALT lymphoma.**

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma arises from mucosal lymphoid tissue that is acquired usually as a reaction to *Helicobacter pylori* infection.[1,2] Growth of these lymphoma cells in culture can be stimulated by strain-specific heat-killed *H. pylori*.[3,4] This response is mediated by intratumoral *H. pylori*–specific T cells and involves CD40 and CD40L costimulatory molecules.[3,4] Intriguingly, the lymphoma immunoglobulin recognizes a variety of autoantigens without cross-reactivity with *H. pylori*.[5,6] In the clinical setting, eradication of *H. pylori* leads to complete regression of gastric MALT lymphoma in approximately 75% of cases.[7–13]

The time for regression to take place after *H. pylori* eradication varies from a few weeks to 18 months.[7–13] Therefore, prolonged follow-up with repeated endoscopy and gastric biopsies is essential to determine whether a lymphoma responds to *H. pylori* eradication or requires additional therapy. The prognostic value of clinical staging has been extensively examined with the help of endoscopic ultrasonography, which allows assessment of the extent of tumor invasion to the gastric wall and regional lymph nodes.[11–13] In general, stage II_E or above lymphomas, in which gastric lymph nodes or adjacent or

Abbreviations used in this paper: CR, complete remission; MALT, mucosa-associated lymphoid tissue; NR, nonresponsive; RT-PCR, reverse-transcription polymerase chain reaction.

0016-5085/02/$35.00
doi:10.1053/gast.2002.33047

remote organs are involved, do not respond to *H. pylori* eradication.[11–13] In stage I_E cases, in which tumors are confined to the gastric wall, staging has limited value in prediction of the response, although tumors that involve the muscularis propria or serosa (stage I_{E2}) have a higher failure rate than those restricted to the submucosa (stage I_{E1}).[11–13] However, most gastric MALT lymphomas are at stage I_E at diagnosis, and alternative prognostic markers are therefore needed.

t(11;18)(q21;q21) occurs specifically in MALT lymphomas and is the most frequent genetic abnormality found in this tumor, detected in about 40% of gastric cases.[14–26] The translocation fuses the amino terminal of the *API2* gene to the carboxyl terminal of the *MALT1* gene and generates a chimeric fusion product.[16–18] In in vitro assays, the API2-MALT1 fusion product activates nuclear factor κB,[27,28] a transcription factor for several survival-related genes, including those encoding cytokines, growth factors, cell adhesion molecules, and several cell apoptosis inhibitors.[29] Thus, it is most likely that the fusion product confers a survival advantage to MALT lymphoma cells. In keeping with this, t(11;18)(q21;q21) is significantly associated with more advanced gastric MALT lymphomas.[26,30,31] In a small series of cases, we showed that t(11;18)(q21;q21)-positive MALT lymphomas failed to respond to *H. pylori* eradication.[25] However, this finding remains to be validated in a large cohort. More importantly, it is unknown whether the translocation predicts the response of stage I_E lymphoma to *H. pylori* eradication. We have developed a novel reverse-transcription polymerase chain reaction (RT-PCR) method for detection of t(11;18)(q21;q21) from archival formalin-fixed and paraffin-embedded tissues and addressed these issues in a large cohort of cases collected from 5 European lymphoma centers.

Materials and Methods

Patients and Materials

A series of 111 patients with *H. pylori*–positive gastric MALT lymphoma who were treated with antibiotics alone was retrospectively recruited from the Groupe d'Etude des Lymphomes Digestifs, France (33 cases); Department of Pathology, The Netherlands Cancer Institute, The Netherlands (32 cases); Servizi di Anatomia Patologica e Gastroenterologia, Università degli Studi di Bologna, Italy (24 cases); the German MALT Lymphoma Study Group (18 cases); and Department of Histopathology, University College London (4 cases). The selection of patients was biased toward those who showed no response to *H. pylori* eradication, and the proportion of *H. pylori* eradication nonresponsive (NR) cases in different groups was similar. The diagnosis of gastric MALT lymphoma was made according to histologic criteria described by Isaacson et al.[9,32] In all cases, there was dense diffuse infiltrate of centrocyte-like cells in lamina propria with prominent lymphoepithelial lesions. Clinical staging according to the Ann Arbor system modified by Musshoff was performed in each case before therapy.[33] In 64 cases, the extent of lymphoma invasion of the gastric wall and regional lymph nodes was determined by endoscopic ultrasonography, which allows further division of stage I_E tumors into I_{E1} (restricted to the submucosa) and I_{E2} (extended to the muscularis or serosa).[11] *H. pylori* eradication was achieved by administration of a 2-week course of amoxicillin (3 × 750 mg daily) and omeprazole (3 × 40 mg daily).[7,10,12] One month after completing the antibiotic therapy, the first gastric endoscopy and biopsy were performed to detect *H. pylori* infection by histology, culture, and PCR of the *H. pylori*–associated urease gene and tumor regression by histology and molecular analysis. These investigations were repeated every 3–4 months until lymphoma showed complete regression or was judged as NR. After achieving complete regression, patients were examined further every 6 months. Lymphomas showing both complete endoscopic and histologic regression were regarded as complete remission (CR). Those that failed to show histologic regression 12 months after successful eradication of *H. pylori* or progressed during follow-up were judged as NR.

Tissue specimens from diagnostic biopsy specimens, including frozen tissues from 22 patients and formalin-fixed and paraffin-embedded tissues from 89 patients, were retrieved for molecular analysis. Where indicated, follow-up biopsy specimens were also analyzed.

RNA Extraction

For frozen tissues, total RNA was extracted from up to 10 mg tissue using the RNeasy Mini Kit (Qiagen, West Sussex, England).

For formalin-fixed and paraffin-embedded tissues, total RNA was extracted using an Ambion RNA isolation kit (AMS Biotechnology, Oxon, England, United Kingdom). Briefly, 5–10 5-μm paraffin sections were deparaffinized in xylene. The tissue was digested with proteinase K (1 mg/mL) for 2 hours at 45°C and solubilized in a guanidinium-based buffer. RNA was extracted with acid phenol/chloroform and precipitated in isopropanol. The precipitated RNA was washed in 75% ethanol and redissolved in 20 μL RNA Storage Solution (AMS Biotechnology).

Detection of t(11;18)(q21;q21) by RT-PCR

The synthesis of complementary DNA (cDNA) and PCR detection of the API2-MALT1 fusion transcript from frozen tissues was performed as described previously.[25,26] Briefly, up to 2 μg total RNA was reverse transcribed into cDNA using the SuperScript Preamplification System (Invitrogen Ltd., Paisley, Scotland, United Kingdom) and oligo(dT) primer. The API2-MALT1 fusion transcript was amplified by PCR using a pair of primers (f-S and f-AS) that covered all the known breakpoints (Table 1 and Figure 1).[26] As control, a 257–base pair fragment of the glucose-6-phosphate dehydro-

Table 1. Primers for RT-PCR of the API2-MALT1 Fusion Transcript and Glucose-6-Phosphate Dehydrogenase

Tissue type	Gene target	Primer	Primer sequence	Expected major PCR products (base pairs)[a]
Frozen tissue	API2-MALT1[b]	Sense	5′-ACA TTC TTT AAC TGG CCC TC	669; 730; 1006; 1279
		Anti-sense	5′-TAG TCA ATT CGT ACA CAT CC	
Paraffin block	G6PD	Sense	5′-GAG GCC GTG TAC ACC AAG ATG AT	258
		Anti-sense	5′-AAT ATA GGG GAT GGG CTT GG	
	API2-MALT1	Sense	5′-GGA AGA GGA GAG AGA AAG AGC A	
		Anti-sense 1	5′-CCA AGA CTG CCT TTG ACT CT	83
		Anti-sense 2	5′-GGA TTC AGA GAC GCC ATC AA	67; 340
		Anti-sense 3	5′-CAA AGG CTG GTC AGT TGT TT	73; 100; (133; 197; 230); 409
	G6PD	Sense	5′-ACG-GCA ACA GAT ACA AGA AC	87
		Anti-sense	5′-CGA AGT GCA TCT GGC TCC	

G6PD, glucose-6-phosphate dehydrogenase.
[a]Alternative splice variants are shown in parentheses.
[b]Gene sequence used for primer design: *API2,* NM_001165; *MALT1,* AF130356; *G6PD,* X55448.1 and M12996.

genase gene spanning 2 exons was amplified in parallel (Table 1). PCR products were analyzed on 0.9% agarose gels by electrophoresis.

For paraffin-embedded samples, cDNA was synthesized using the SuperScript Preamplification System with the following modifications. A mixture of gene-specific primers comprising 1 pmol each of the 3 MALT1 antisense primers (p-AS1, p-AS2, and p-AS3) and the glucose-6-phosphate dehydrogenase antisense primer (Table 1 and Figure 1) was used. In addition, the temperature for primer annealing and cDNA synthesis was at 50°C, rather than 42°C as used for reverse transcription with oligo(dT) primer. To amplify the API2-MALT1 fusion product, primers were designed to flank a short segment of the fusion junction and hence were suitable for amplification of small fragments of cDNA typically prepared from RNA isolated from paraffin-embedded tissues. Three sets of PCR primers were designed: a common *API2* sense primer (p-S) that covered 93% of the known *API2* breakpoints and 3 antisense primers that targeted all 4 variable breakpoints on the *MALT1* gene (Table 1 and Figure 1).[16–26] A separate set of primers was designed for RT-PCR of the glucose-6-phosphate dehydrogenase gene (Table 1 and Figure 1). The size of fragments amplified with these primer pairs is shown in Table 1. PCR was performed separately with each primer pair using a "hot-start touch-down" program.[26] PCR products were analyzed by electrophoresis on 10% polyacrylamide gels. In each case, RT-PCR analysis for API2-MALT1 fusion transcript was performed at least twice in independent experiments.

To validate the efficiency of the RT-PCR system for detection of the API2-MALT1 fusion transcript from paraffin-embedded tissues, we first evaluated the system on 20 t(11;18)(q21;q21)-positive and 10 negative cases that had both frozen and paraffin-embedded tissues and the frozen tissues had been examined for t(11;18) by RT-PCR as described previously.[25,26] The RT-PCR system developed for paraffin-embedded tissues detected the translocation in each of the 20 positive cases and did not show any nonspecific bands. PCR products representing different API2-MALT1 fusions were confirmed by sequencing. To further determine whether the system can be applied to tissues from small biopsy specimens, we performed RT-PCR on microdissected cells from 3 t(11;18)(q21;q21) cases and the translocation was detected in each occasion. Having established the reliability of the system, we screened unknown cases for t(11;18)(q21;q21). The molecular detection of t(11;18)(q21;q21) was performed blindly without knowledge of the clinical follow-up.

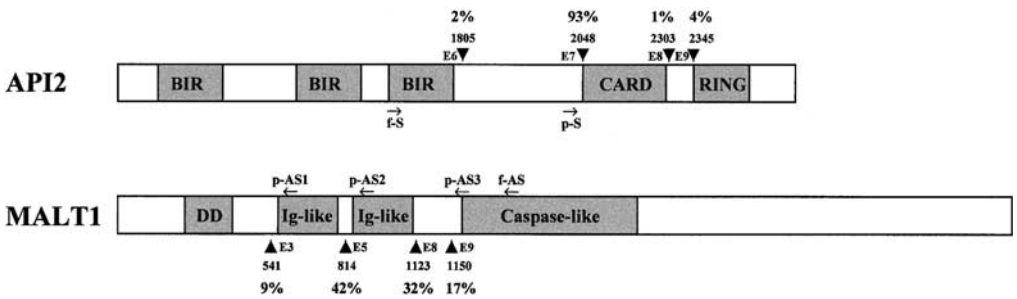

Figure 1. Schematic representation of the *API2* and *MALT1* gene structure and primer positions. Known breakpoints are indicated by *arrowheads*, and nucleic acids are numbered according to cDNA sequence of the *API2* (GeneBank, NM_001165) and *MALT1* gene (AF130356). The frequency of individual known breakpoints was given.[14–26] *Arrows* indicate the position of primers used. f-S and f-AS, sense and anti-sense primer for PCR from frozen tissue; p-S and p-AS, sense and anti-sense primer for PCR from paraffin-embedded tissues; BIR, baculovirus IAP repeat; CARD, caspase recruitment domain; DD, death domain.[21,23]

Sequencing of PCR Products

Where indicated, PCR products were gel purified (QIAquick Gel Extraction Kit; Qiagen) and sequenced in both directions using dRhodamine dye terminators on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA).

Statistical Analysis

χ^2 and Fisher exact tests were used to analyze the correlation between the response of MALT lymphomas to *H. pylori* eradication and clinical staging or t(11;18)(q21;q21) status.

Results

Clinical Staging Predicts Treatment Failure to *H. pylori* Eradication in Stage II_E or Above but Not Stage I_E Gastric MALT Lymphoma

A total of 111 patients with gastric MALT lymphoma were included in the present multicenter study (67 men and 44 women; mean age, 58 years [range, 25–88 years]). *H. pylori* infection was successfully cured in all cases as confirmed by histology and culture of gastric biopsy specimens taken after completion of the antibiotic therapy. After *H. pylori* eradication, patients were followed up by repeated endoscopy and biopsy. The mean period between *H. pylori* eradication and achievement of CR or commencement of other treatment in NR patients was 12 months (range, 1–75 months), and the mean follow-up period to date is 35 months (range, 9–85 months). During follow-up, 48 cases showed CR, whereas 63 cases displayed NR. There is no difference in age and sex between the CR and NR groups. Both groups had similar length of follow-up. Histologically, focal transformed high-grade components were seen in 3 NR but not in any of the CR cases. In the CR group, 2 of 48 cases showed tumor relapse; in both cases, the lymphoma harbored t(11;18) (detailed in the next section).

Among the 48 CR cases, 47 were at stage I_E and 1 at stage II_E. The stage II_E CR case is 1 of 2 that showed lymphoma relapse. Of the 63 NR cases, 20 were at stage II_E or above and the remaining 43 cases were at stage I_E. Despite the fact that most of the lymphomas at stage II_E or above (20 of 21 [95%]) did not respond to *H. pylori* eradication ($P < 0.001$), almost one half of stage I_E tumors also did not respond to *H. pylori* eradication (43 of 90 [48%], $P > 0.05$). Therefore, the staging failed to predict the response of stage I_E gastric MALT lymphoma to *H. pylori* eradication. Among cases with stage I_E lymphoma, there was no difference in age, sex, and follow-up periods between the CR and NR groups ($P > 0.05$) (Table 2). The extent of lymphoma invasion within the gastric wall was assessed by endoscopic ultrasonography in 64 cases with stage I_E lymphoma. There was no difference in the response of gastric MALT lymphoma to *H. pylori* eradication between cases showing stage I_{E1} and stage I_{E2} disease ($P > 0.05$) (Table 2).

Table 2. Clinical and Histopathologic Features of Stage I_E Gastric MALT Lymphomas and Their Responses to *H. pylori* Eradication Therapy

	Complete regression	No regression
Number of patients	47	43
Age (*yr*)		
Mean	60	57
Range	25–85	30–88
Sex		
M	29	21
F	18	22
Histology with high grade component	0	3
Stages by endoscopic ultrasonography		
I_{E1}	29	30
I_{E2}	3	2
Follow-up period (*mo*)		
Intervals[a]		
Mean	8.2	15
Range	1–26	5–75
Follow-up to date		
Mean	38	30
Range	10–82	9–85

[a]The time between *H. pylori* eradication and complete regression or commencement of other treatment in NR cases.

t(11;18)(q21;q21) Is a Marker for Gastric MALT Lymphomas That Do Not Respond to *H. pylori* Eradication, Including Those at Stage I_E

All cases presented in this study showed successful RT-PCR of the reference gene glucose-6-phosphate dehydrogenase. The API2-MALT1 fusion PCR product varied in size depending on the breakpoints and primer sets used, but accurate sizing of the PCR product on polyacrylamide gels and the characteristic PCR patterns allowed detection of t(11;18)(q21;q21) with high confidence (Figure 2). In 13 cases, PCR bands were weak and sequencing confirmation was performed. Overall, t(11;18) was positive in 40% (44 of 111) of cases detected. The positivity of t(11;18) detected from frozen tissues (9 of 22 cases [41%]) was similar to that from paraffin-embedded tissues (35 of 89 cases [39%]) ($P > 0.05$). The combined results from both frozen and paraffin-embedded tissues are summarized as follows.

Of the 48 CR cases, 2 were t(11;18)(q21;q21) positive (Figure 3). One of these 2 cases, a stage I_E tumor,

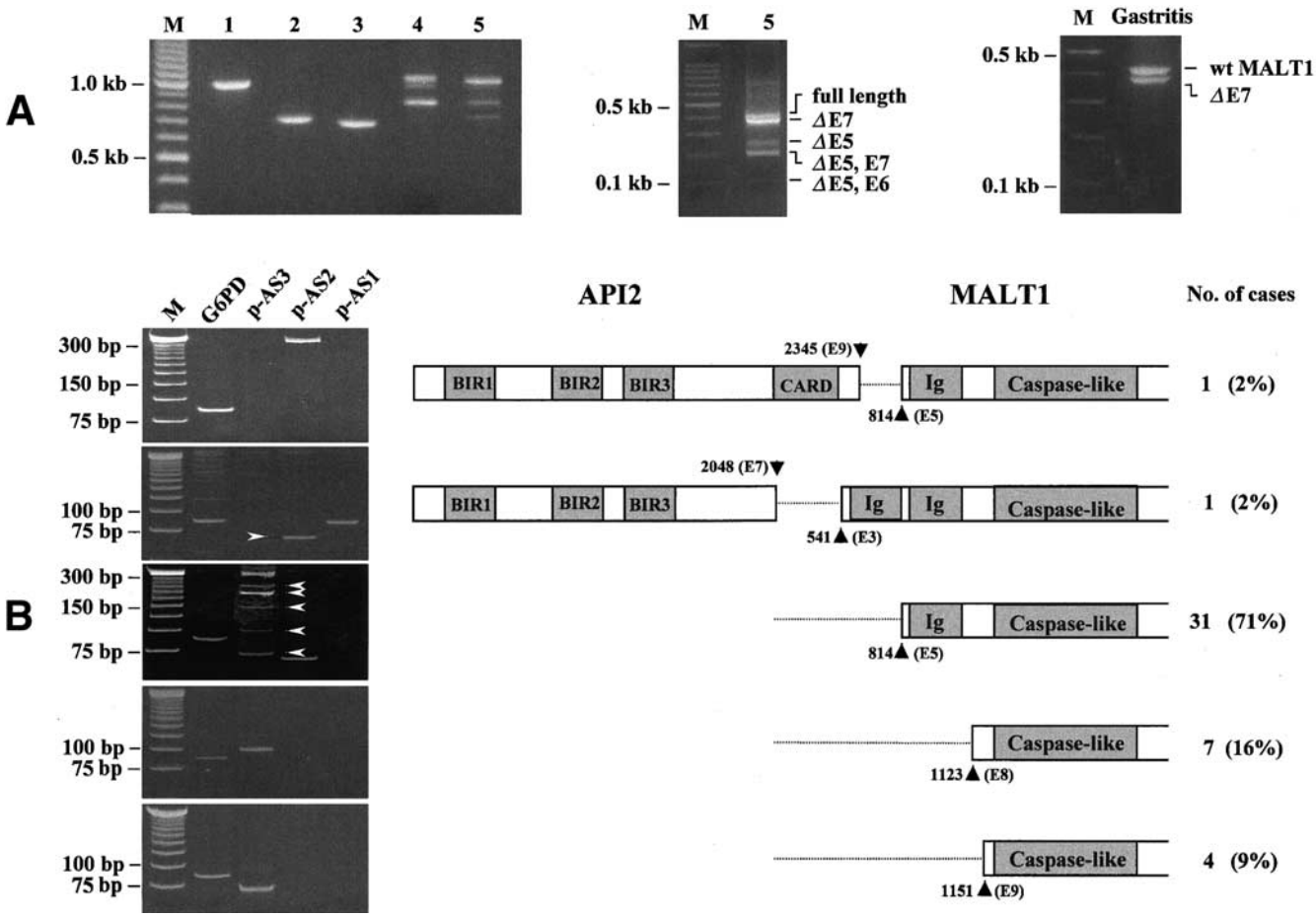

Figure 2. Detection of the API2-MALT1 fusion transcript by RT-PCR. (*A*) *Left panel*: examples of RT-PCR from frozen tissue samples using primer f-S and f-AS. Cases 4 and 5 harbor an API2-MALT1 fusion with breakpoint immediately upstream of exon 5 of the *MALT1* gene and show alternative splice variants of the fusion transcript. M, molecular-weight marker. *Middle panel*: RT-PCR with primer p-S and f-AS in case 5 shows alternative splice variants of the API2-MALT1 fusion. Deleted exons are indicated. *Right panel*: RT-PCR of the *MALT1* gene from a gastritis tissue shows a major splice variant with deletion of exon 7. wt, wild type. (*B*) Examples of various API2-MALT1 fusion transcripts detected from paraffin-embedded tissues. PCR products derived from primers p-AS2 or p-AS3 show splice variants, which are indicated by *arrowheads*. Representative fusion products are illustrated schematically, and their breakpoint and frequency of occurrence are shown.

achieved CR 25 months after *H. pylori* eradication. The remission lasted for 56 months, but tumor reoccurred later in the absence of *H. pylori* reinfection. PCR of the rearranged immunoglobulin gene confirmed the clonal lineage between the original lymphoma and the recurrence. t(11;18)(q21;q21) was detected only in follow-up biopsy specimens showing the tumor relapse but not in those that showed CR. The other t(11;18)(q21;q21)-positive case was a stage II_E tumor, in which CR was achieved 9 months after *H. pylori* eradication, and the remission has been maintained so far for 32 months. However, t(11;18)(q21;q21) was detected in the last follow-up biopsy specimen. A review of histology of the biopsy specimen showed a small crushed fragment of lymphoid tissue suspicious of tumor relapse. *H. pylori* was not seen.

In contrast to the CR group, 42 of the 63 NR cases (67%) were positive for the translocation, including 26 of the 43 stage I_E tumors (60%) (Figure 3). Thus, t(11;18)(q21;q21) could predict the response of most early gastric MALT lymphomas to *H. pylori* eradication ($P < 0.001$). As expected, the frequency of t(11;18)(q21;q21) was much higher in lymphomas at stage II_E or above (16 of 20 [80%]) than those at stage I_E ($P < 0.001$) (Figure 3).

Because the API2-MALT1 fusion products with intact Ig-C2 domains are more potent activators of nuclear factor κB than those without Ig-C2 domains[27] and therefore may be more oncogenic,[28] we correlated the type of API2-MALT1 fusion with clinical staging. Of the 44 t(11;18)(q21;q21)-positive cases, 33 fusion transcripts had 1 or 2 intact Ig-C2 domains, whereas 11 did not contain Ig-C2 domains (Figure 2). Tumors bearing the fusion product with intact Ig-C2 domains (14 of 33 [42%]) were more often at stage II_E or above than those harboring the fusion product without Ig-C2 domains (3

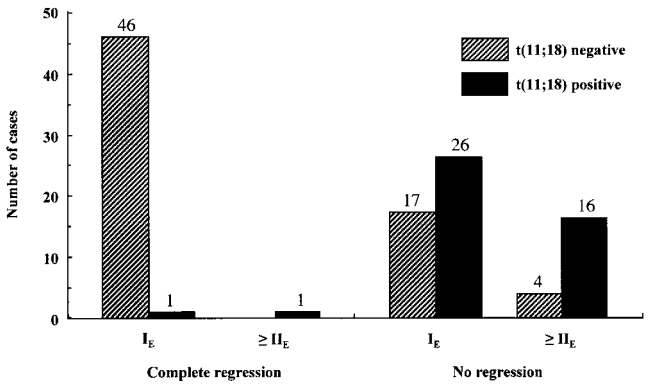

Figure 3. Correlation between response of gastric MALT lymphoma to *H. pylori* eradication therapy and clinical staging and presence of t(11;18)(q21;q21). Clinical staging has little value in predication of the response of stage I_E gastric MALT lymphoma to *H. pylori* eradication therapy. In contrast, the translocation can predict 60% of *H. pylori* therapy NR cases at stage I_E.

of 11 [27%]), although statistical analysis did not show any significant difference ($P > 0.05$).

Alternative Splice Variants of the API2-MALT1 Fusion Transcript

The breakpoint in the *MALT1* gene occurred variably immediately upstream of 4 exons (3, 5, 8, and 9), whereas the breakpoint in the *API2* gene was always immediately downstream of exon 7 with an exception in 1 case that occurred after exon 9. Various API2-MALT1 fusions gave different PCR patterns (Figure 2). When the breakpoint occurred immediately before exons 8 and 9 of the *MALT1* gene, RT-PCR showed only a single band. When the breakpoint occurred before exon 5, 1 expected band together with 4 additional smaller bands were seen. These additional PCR bands were of variable intensity but weaker than the expected fusion product. Sequencing of these bands confirmed that they were API2-MALT1 fusions identical to the corresponding fusion product in each case but with deletion of 1 or more exons of the *MALT1* gene. It is most likely that these additional PCR bands represent alternative splice variants of the fusion transcript.

The splice variants of the API2-MALT1 fusion product are best shown by PCR from frozen tissues with the sense primer positioned just upstream of the *API2* breakpoint (p-S), which yielded smaller fusion products and hence gave better separation on gels. Case 5, which harbored an API2-MALT1 fusion transcript with breakpoint immediately before exon 5 of the *MALT1* gene, showed 4 additional bands (Figure 2). They represented alternative splice variants with deletion of exon 7, exon 5, exons 5 and 7, and exons 5 and 6 of the *MALT1* gene. The variant without exon 7 did not alter the amino acid reading frame and represented the major splice variant, whereas other splice species were minor and those with deletion of exon 5 or both exons 5 and 7 introduced a stop codon.

To examine whether these alternative splice events occur in wild-type *MALT1*, RT-PCR of the *MALT1* gene was performed. In contrast to the API2-MALT1 fusion, only a splice variant with exon 7 deletion was found in *MALT1* (Figure 2). However, in keeping with the splice variants of the fusion transcript, the MALT1 transcript with exon 7 deletion was a major type (Figure 2).[3,4]

Discussion

H. pylori eradication leads to complete regression of gastric MALT lymphoma in 75% of cases and is widely accepted as the first-line treatment for this tumor.[7–13] One of the major dilemmas in clinical management of patients with this disease is the identification of those that will not respond to *H. pylori* eradication and require chemotherapy or radiotherapy. At present, this requires prolonged follow-up with repeated endoscopy and gastric biopsy. Clinical staging is helpful in predicting the response because lymphomas at stage II_E or above rarely respond to *H. pylori* eradication. However, the predictive value of clinical staging for stage I_E tumors is limited[11–13] and better prognostic markers are needed. We have shown that t(11;18)(q21;q21) is a marker for NR gastric MALT lymphomas, including those at stage I_E. In the stage I_E cases, the translocation allows this prediction in 60% of NR cases. None of the CR cases were positive for t(11;18)(q21;q21), with the exception of the 2 equivocal cases described.

Our findings indicate that t(11;18)(q21;q21)-positive gastric MALT lymphomas do not undergo regression after *H. pylori* eradication and require other conventional therapies up front. Nevertheless, *H. pylori* should be eradicated in all cases because this not only eliminates reactive lymphoid infiltrates but most likely has an adjuvant effect because in vitro experiments have shown that *H. pylori* stimulates t(11;18)(q21;q21)-positive lymphoma cells to proliferate via T-cell help.[3,4] Moreover, eradication of *H. pylori* and reactive lymphoid infiltrates may reduce the risk of developing secondary tumors in the stomach.

Among the NR cases, 33% failed to show t(11;18)(q21;q21) by RT-PCR. Our RT-PCR strategy for frozen tissues would theoretically detect 100% of known breakpoints in both the *API2* and *MALT1* genes. However, the RT-PCR methodology for paraffin-embedded tissues would miss some of the 3 minor *API2* break-

points, which account for 7% of the total API2-MALT1 fusions.[16–26] Thus, our current results may slightly underestimate the true frequency of t(11;18)(q21;q21). For prospective clinical screening, PCR with primers for these minor breakpoints should be included and multiplex amplification in a single tube may offer a practical approach.[35]

In about 25% of cases, resistance of gastric MALT lymphoma to *H. pylori* eradication seems to be caused by other factors. MALT lymphomas with chromosomal translocation involving the *BCL10* locus, such as t(1;14)(p22;q32)[36,37] and t(1;2)(p22;p12),[38] are typically those at advanced stages and are unlikely to respond to *H. pylori* eradication.[39] Lymphomas bearing these translocations can be detected immunohistochemically by strong BCL10 nuclear expression.[40] *H. pylori*–associated gastric MALT lymphoma in patients with autoimmune disease has been shown to be resistant to antibiotic treatment.[41] The *fas* gene is frequently mutated in MALT lymphoma in patients with autoimmunity,[42] and *fas* gene mutations may confer resistance of gastric MALT lymphoma to *H. pylori* eradication.

The breakpoints in the *API2* gene are always downstream of the third BIR domain but upstream of the carboxyl RING, whereas the breakpoints in the *MALT1* gene are consistently upstream of the carboxyl caspase-like domain. Thus, the resulting API2-MALT1 fusion transcripts always comprise the amino terminal *API2* with 3 intact BIR domains and the carboxyl terminal *MALT1* region containing an intact caspase-like domain. The specific selection of these domains of the *API2* and *MALT1* gene to form a fusion product strongly suggests their importance and synergy in oncogenesis. The BIR domain of API2 has been shown to be antiapoptotic.[43] However, the antiapoptotic activity of the API2 BIR domain was weak and has been shown to be suppressed by its C-terminal RING finger domain.[43] As a result, wild-type API2 did not protect cells from apoptosis on stimulation by death signals.[43] The negative effect of the RING finger on BIR function may be associated with its ability to promote autoubiquitination and degradation.[43,44] Replacement of the C-terminal of API2 with the C-terminal of MALT1 by the fusion product would release the intrinsic antiapoptotic activity of the BIR domain and therefore make the new molecule antiapoptotic. Indeed, the API2-MALT1 fusion product, but not API2 or MALT1 alone, has been shown to activate nuclear factor κB, and the caspase-like domain is required for this function.[27] Moreover, the fusion products with intact Ig-C2 domains are more potent activators of nuclear factor κB than those without Ig-C2 domains.[27,28] In keeping with this, we found that tumors bearing the fusion product with 1 or 2 intact Ig-C2 domains were more often at stage II_E or above than those harboring the fusion without the Ig-C2 domain. Alternative splice variants of the API2-MALT1 fusion transcript are present, but their functional significance is unclear.

References

1. Wotherspoon AC, Ortiz Hidalgo C, Falzon MR, Isaacson PG. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 1991;338:1175–1176.
2. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
3. Hussell T, Isaacson PG, Crabtree JE, Spencer J. The response of cells from low-grade B-cell gastric lymphomas of mucosa-associated lymphoid tissue to *Helicobacter pylori*. Lancet 1993;342:571–574.
4. Hussell T, Isaacson PG, Crabtree JE, Spencer J. *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 1996;178:122–127.
5. Hussell T, Isaacson PG, Crabtree JE, Dogan A, Spencer J. Immunoglobulin specificity of low grade B cell gastrointestinal lymphoma of mucosa-associated lymphoid tissue (MALT) type. Am J Pathol 1993;142:285–292.
6. Greiner A, Marx A, Heesemann J, Leebmann J, Schmausser B, Muller-Hermelink HK. Idiotype identity in a MALT-type lymphoma and B cells in *Helicobacter pylori* associated chronic gastritis. Lab Invest 1994;70:572–578.
7. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. 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.
8. Roggero E, Zucca E, Pinotti G, Pascarella A, Capella C, Savio A, Pedrinis E, Paterlini A, Venco A, Cavalli F. Eradication of *Helicobacter pylori* infection in primary low-grade gastric lymphoma of mucosa-associated lymphoid tissue. Ann Intern Med 1995;122:767–769.
9. Savio A, Franzin G, Wotherspoon AC, Zamboni G, Negrini R, Buffoli F, Diss TC, Pan L, Isaacson PG. 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.
10. Thiede C, Morgner A, Alpen B, Wundisch T, Herrmann J, Ritter M, Ehninger G, Stolte M, Bayerdorffer E, Neubauer A. What role does *Helicobacter pylori* eradication play in gastric MALT and gastric MALT lymphoma? Gastroenterology 1997;113:S61–S64.
11. Sackmann M, Morgner A, Rudolph B, Neubauer A, Thiede C, Schulz H, Kraemer W, Boersch G, Rohde P, Seifert E, Stolte M, Bayerdoerffer E. Regression of gastric MALT lymphoma after eradication of *Helicobacter pylori* is predicted by endosonographic staging. MALT Lymphoma Study Group. Gastroenterology 1997;113:1087–1090.
12. Ruskone-Fourmestraux A, Lavergne A, Aegerter PH, Megraud F, Palazzo L, de Mascarel A, Molina T, Rambaud JL. Predictive factors for regression of gastric MALT lymphoma after anti-*Helicobacter pylori* treatment. Gut 2001;48:297–303.
13. Nakamura S, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, Yao T, Tsuneyoshi M, Iida M, Fujishima M. Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of *Helicobacter pylori*. Gut 2001;48:454–460.
14. Auer IA, Gascoyne RD, Connors JM, Cotter FE, Greiner TC, Sanger

WG, Horsman DE. t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. Ann Oncol 1997;8:979–985.
15. Ott G, Katzenberger T, Greiner A, Kalla J, Rosenwald A, Heinrich U, Ott MM, Muller Hermelink HK. The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not high-grade malignant non-Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT-) type. Cancer Res 1997;57:3944–3948.
16. Dierlamm J, Baens M, Wlodarska I, Stefanova-Ouzounova M, Hernandez JM, Hossfeld DK, De Wolf-Peeters C, Hagemeijer A, Van den Berghe H, Marynen P. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. Blood 1999;93:3601–3609.
17. Akagi T, Motegi M, Tamura A, Suzuki R, Hosokawa Y, Suzuki H, Ota H, Nakamura S, Morishima Y, Taniwaki M, Seto M. A novel gene, MALT1 at 18q21, is involved in t(11;18) (q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. Oncogene 1999;18:5785–5794.
18. Morgan JA, Yin Y, Borowsky AD, Kuo F, Nourmand N, Koontz JI, Reynolds C, Soreng L, Griffin CA, Graeme-Cook F, Harris NL, Weisenburger D, Pinkus GS, Fletcher JA, Sklar J. Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 in chromosome 18. Cancer Res 1999;59:6205–6213.
19. Remstein ED, James CD, Kurtin PJ. Incidence and subtype specificity of API2-MALT1 fusion translocations in extranodal, nodal, and splenic marginal zone lymphomas. Am J Pathol 2000;156: 1183–1188.
20. Baens M, Maes B, Steyls A, Geboes K, Marynen P, De Wolf-Peeters C. 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.
21. Dierlamm J, Baens M, Stefanova-Ouzounova M, Hinz K, Wlodarska I, Maes B, Steyls A, Driessen A, Verhoef G, Gaulard P, Hagemeijer A, Hossfeld DK, De Wolf-Peeters C, Marynen P. Detection of t(11;18)(q21;q21) by interphase fluorescence in situ hybridization using API2 and MLT specific probes. Blood 2000; 96:2215–2218.
22. Motegi M, Yonezumi M, Suzuki H, Suzuki R, Hosokawa Y, Hosaka S, Kodera Y, Morishima Y, Nakamura S, Seto M. API2-MALT1 chimeric transcripts involved in mucosa-associated lymphoid tissue type lymphoma predict heterogeneous products. Am J Pathol 2000;156:807–812.
23. Nakamura T, Nakamura S, Yonezumi M, Suzuki T, Matsuura A, Yatabe Y, Yokoi T, Ohashi K, Seto M. Helicobacter pylori and the t(11;18)(q21;q21) translocation in gastric low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type. Jpn J Cancer Res 2000;91:301–309.
24. Kalla J, Stilgenbauer S, Schaffner C, Wolf S, Ott G, Greiner A, Rosenwald A, Dohner H, Muller-Hermelink HK, Lichter P. Heterogeneity of the API2-MALT1 gene rearrangement in MALT-type lymphoma. Leukemia 2000;14:1967–1974.
25. Liu H, Ruskone-Fourmestraux A, Lavergne-Slove A, Ye H, Molina T, Bouhnik Y, Hamoudi RA, Diss TC, Dogan A, Megraud F, Rambaud JC, Du M-Q, Isaacson PG. Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy. Lancet 2000;357:39–40.
26. Liu H, Ye H, Dogan A, Ranaldi R, Hamoudi RA, Bearzi I, Isaacson PG, Du M-Q. T(11;18)(q21;q21) is associated with more advanced MALT lymphoma that expresses nuclear BCL10. Blood 2001;98:1182–1187.
27. Uren GA, O'Rourke K, Aravind L, Pisabarro TM, Seshagiri S, Koonin VE, Dixit MV. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. Mol Cell 2000;6:961–967.
28. Lucas PC, Yonezumi M, Inohara N, McAllister-Lucas LM, Abazeed ME, Chen FF, Yamaoka S, Seto M, Nunez G. 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.
29. Bours V, Bentires-Alj M, Hellin AC, Viatour P, Robe P, Delhalle S, Benoit V, Merville MP. Nuclear factor-kappa B, cancer, and apoptosis. Biochem Pharmacol 2000;60:1085–1089.
30. Alpen B, Neubauer A, Dierlamm J, Marynen P, Thiede C, Bayerdorfer E, Stolte M. Translocation t(11;18) absent in early gastric marginal zone B-cell lymphoma of MALT type responding to eradication of *Helicobacter pylori* infection. Blood 2000;95:4014–4015.
31. Sugiyama T, Asaka M, Nakamura S, Yonezumi S, Seto M. API2-MALT1 chimeric transcript is a predictive marker for the responsiveness of *H. pylori* eradication treatment in low-grade gastric MALT lymphoma. Gastroenterology 2001;120:1884–1885.
32. Isaacson PG, Norton AJ. Extranodal lymphomas. Edinburgh: Churchill Livingstone, 1994.
33. Musshoff K. Klinische stadieneinteilung der nicht-Hodgkin-lymphoma. Strahlentherapie 1977;153:218–221.
34. Baens M, Steyls A, Dierlamm J, De Wolf-Peeters C, Marynen P. Structure of the MLT gene and molecular characterization of the genomic breakpoint junctions in the t(11;18)(q21;q21) of marginal zone B-cell lymphomas of MALT type. Genes Chromosomes Cancer 2000;29:281–291.
35. Inagaki H, Okabe M, Seto M, Nakamura S, Ueda R, Eimoto T. 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.
36. Willis TG, Jadayel DM, Du MQ, Peng H, Perry AR, Abdul-Rauf M, Price H, Karran L, Majekodunmi O, Wlodarska I, Pan L, Crook T, Hamoudi R, Isaacson PG, Dyer MJ. Bcl10 is involved in t(1; 14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. Cell 1999;96:35–45.
37. Zhang Q, Siebert R, Yan M, Hinzmann B, Cui X, Xue L, Rakestraw KM, Naeve CW, Beckmann G, Weisenburger DD, Sanger WG, Nowotny H, Vesely M, Callet-Bauchu E, Salles G, Dixit VM, Rosenthal A, Schlegelberger B, Morris SW. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). Nat Genet 1999;22:63–68.
38. Achuthan R, Bell SM, Leek JP, Roberts P, Horgan K, Markham AF, Selby PJ, MacLennan KA. Novel translocation of the BCL10 gene in a case of mucosa associated lymphoid tissue lymphoma. Genes Chromosomes Cancer 2000;29:347–349.
39. Du M-Q, Peng HZ, Liu H, Hamoudi RA, Diss TC, Willis TG, Ye HT, Dogan A, Wotherspoon AC, Dyer MJS, Isaacson PG. BCL10 mutation in lymphoma. Blood 2000;95:3885–3890.
40. Ye H, Dogan A, Karran L, Willis TG, Chen L, Wlodarska I, Dyer MJ, Isaacson PG, Du MQ. BCL10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma. Am J Pathol 2000;157:1147–1154.
41. Raderer M, Osterreicher C, Machold K, Formanek M, Fiebiger W, Penz M, Dragosics B, Chott A. Impaired response of gastric MALT-lymphoma to *Helicobacter pylori* eradication in patients with autoimmune disease. Ann Oncol 2001;12:937–939.
42. Gronbaek K, Straten PT, Ralfkiaer E, Ahrenkiel V, Andersen MK, Hansen NE, Zeuthen J, Hou-Jensen K, Guldberg P. Somatic Fas mutations in non-Hodgkin's lymphoma: association with extranodal disease and autoimmunity. Blood 1998;92:3018–3024.
43. Clem RJ, Sheu TT, Richter BW, He W-W, Thornberry NA, Duckett CS, Hardwick JM. C-IAP1 is cleaved by caspases to produce a

pro-apoptotic c-terminal fragment. J Biol Chem 2001;276:7602–7608.
44. Yang Y, Fang S, Jensen JP, Weissman AM, Ashwell JD. Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. Science 2000;288:874–877.

Received October 24, 2001. Accepted January 24, 2002.

Address requests for reprints to: Ming-Qing Du, Ph.D., Department of Histopathology, Royal Free and University College Medical School, University College London, Rockefeller Building, University Street, London WC1E 6JJ, England. e-mail: m.du@ucl.ac.uk; fax: (44) 20-7387-3674.

Supported by research grants from the Leukemia Research Fund, Cancer Research Campaign, Délégation à la Recherche Clinique, Assistance Publique-Hôpitaux de Paris, Associazioine Italiana per la Ricerca sul Cancro, and Deutsche Krebshilfe (grant 70-225I).

H.L. and H.Y. contributed equally to this work.

The authors thank J. Audouin, L. Bedenne, O. Bouche, Y. Bouhnik, J. Fournet, A. De Mascarel, Ph. Moreau, J. Lafon, and A. Pariente of the Groupe d'Etude des Lymphomes Digestifs, France, for contribution of part of the specimens used for this study, Dr. Tim C. Diss for critical reading of the manuscript, and M. Sisnaki for technical assistance.