Research letters

Resistance of t(11;18) positive gastric mucosa-associated lymphoid tissue lymphoma to *Helicobacter pylori* eradication therapy

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20–30% of gastric mucosa-associated lymphoid tissue (MALT) lymphoma associated with *Helicobacter pylori* do not regress after antibiotic therapy. Regression can be assessed only by extended follow-up. To assess whether t(11;18, q21;q21), which results in a chimeric transcript between the *API2* and *MLT* genes, predicts lymphoma resistance to antibiotic therapy, we screened for the fusion transcript with RT-PCR in ten responsive and 12 non-responsive gastric MALT lymphomas. The *API2-MLT* transcript was detected in nine (75%) of 12 patients non-responsive to antibiotic therapy but not in responsive patients. Most *H pylori*-associated gastric MALT lymphomas that do not respond to antibiotic therapy are associated with t(11;18, q21;q21).

Gastric B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) arises from mucosal lymphoid tissue that is normally acquired as a reaction to *Helicobacter pylori* infection. The presence of the organism has a profound effect on the lymphoma. Growth of the lymphoma B cells in culture can be stimulated by the addition of heat-killed strain-specific *H pylori*. Surprisingly, lymphoma B-cell immunoglobulin does not recognise *H pylori* antigens but rather various autoantigens. The intratumoral T cells are *H pylori* specific and provide contact-mediated help for the growth of the neoplastic B cells.

Eradication of *H pylori* with antibiotics leads to regression and cure of the lymphoma in 75% of cases.[1,2] After *H pylori* eradication, regression time varies from a few weeks to 18 months, but histology of the gastric biopsy samples cannot predict which lymphomas will respond and which will be resistant to antibiotic therapy. Thus, repeated endoscopy with gastric biopsies is necessary to assess the reaction of the lymphoma to the antibiotic therapy before making the decision to give other conventional treatments in resistant cases.

Findings from cytogenetic studies of gastric MALT lymphoma have shown t(11;18, q21;q21) in about 30% of cases. The breakpoint of this translocation involves rearrangement of the *API2* gene on chromosome 11 and a novel gene, *MLT*, on chromosome 18.[3] Translocation results in a chimeric transcript with the amino terminal of *API2* fusing with the carboxyl terminal of *MLT*, which can be detected by RT-PCR. The *API2-MLT* fusion is thought to give a survival advantage to MALT lymphoma cells. This theory raises the question of whether t(11;18, q21;q21) would account for *H pylori*-independent survival or growth of gastric MALT lymphoma and could, therefore, predict the resistance of the lymphoma to *H pylori* eradication therapy, thus enabling early identification of patients who require chemotherapy.

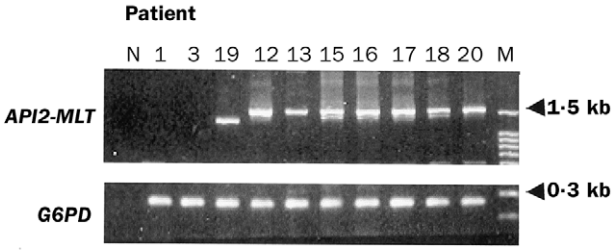
As part of the French prospective multicentre study of the Groupe d' Etude des Lymphomes Digestifs, we obtained fresh-frozen gastric biopsy samples taken at the time of diagnosis from 22 patients with clinically staged gastric MALT lymphoma and known *H pylori* status (table) who were subsequently treated with antibiotics. After successful eradication of the organism, we followed up patients by repeated gastric endoscopy and biopsy for 4·5–60·0 months. Histological changes were carefully reviewed by the panel of pathologists' committee without knowledge of molecular data.

Gastric lymphoma regressed completely after eradication of *H pylori* in nine patients within 3·0–16·0 months, and partly in one patient after 1·5 months, but showed no sign of

Patient number	Sex	Age (years)	*H pylori* serology/histology	Stage*	Clonality	Remission after antibiotic therapy	Months between *H pylori* eradication and remission or other treatment	Other treatment	t(11;18) *API2/MLT*†
1	F	47	+/+	I_E	ND	Partial	1·5	..	..
2	F	62	ND/+	ND	S	Complete	3	..	..
3	F	71	+/+	I_E	S	Complete	6	..	..
4	F	68	−/+	I_E	M	Complete	6	..	..
5	M	76	ND/+	ND	M	Complete	9	..	..
6	M	70	+/−	I_E	M	Complete	11	..	..
7	F	55	+/+	I_E	ND	Complete	12	..	..
8	M	64	+/+	I_E	M	Complete	12	..	..
9	F	25	+/+	I_E	S	Complete	13	..	..
10	M	46	+/+	I_E	M	Complete	16	..	..
11	M	60	+/+	I_E	M	No change	4·5	Gastrectomy	..
12	M	54	−/+	II_E	M	No change	5	Gastrectomy	2048/715
13	F	62	+/+	IV_E	M	No change	6	Gastrectomy	2048/715
14	F	62	+/+	I_E	S	No change	8	Gastrectomy	..
15	M	65	+/+	II_{E1}	M	No change	10	Gastrectomy	2048/715
16	M	61	−/+	II_{E2}	M	No change	10	Gastrectomy	2048/715
17	M	47	+/+	II_E	S	No change	12	Gastrectomy	2048/715
18	M	44	+/+	II_{E1}	M	No change	12	Gastrectomy	2048/715
19	M	49	−/+	I_E	M	No change	12·5	Gastrectomy	2048/991
20	M	62	+/+	I_E	M	No change	19	Gastrectomy	2048/715
21	M	43	+/+	I_E	M	No change	27	Gastrectomy	..
22	M	42	+/+	II_E	M	No change	60	Gastrectomy	2048/715

ND=not done, S=smear, M=monoclonal pattern (monoclonal band can be seen in 80% of MALT lymphoma by PCR-based analysis of rearranged Ig gene heavy-chain genes).
*Ann Arbor staging system; †number denotes the breakpoint in API2 and MLT according to their DNA sequence (GeneBank Accession number: API2, NM_001165; MLT, AB026118).

t(11;18, q21;q21) in *H pylori* eradication responsive and non-responsive gastric MALT lymphomas



Detection of t(11;18, q21;q21) by RT-PCR in gastric MALT lymphoma
N=negative control, M=DNA marker.

endoscopic and histological changes in the remaining 12 patients. Of these 12 patients, eight were followed up for 10·0–60·0 months, judged to be resistant to *H pylori* eradication, and subsequently treated by gastrectomy. The remaining four patients were followed up for 4·5–8·0 months before gastrectomy because of tumour growth or complications (haemorrhage), and were tentatively assumed to be non-responsive to *H pylori* eradication.

RNA was extracted from the primary diagnostic biopsy sample from each patient and also from gastrectomy samples from seven patients with RNeasy Mini Kit (QIAGEN, Crawley, UK). cDNA was synthesised with oligo-dT primers using SUPERSCRIPT First-Strand Synthesis System (GIBCO-BRL, Paisley, UK). PCR for the *API2-MLT* fusion transcript was done with two sets of primers (*API2* 5′-CTGGTGTGAATGACAAGGTC-3′ with either *MLT1* 5′-TAGTCAATTCGTACACATCC-3′ or *MLT2* 5′-CAAAGGCTGGTCAGTTGTTT-3′) in separate reactions, which would detect most known t(11;18, q21;q21) breakpoints. PCR products were sequenced further to identify the breakpoint. We did RT-PCR for glucose-6-phosphate dehydrogenase gene (sense strand primer 5′GAGGCCGTGTACACCAAGAT3′; anti-sense strand primer 5′AATATAGGGGATGGGCTTGG3′) for every patient to control for template quality. Clonality was assessed by PCR analysis of the rearranged immunoglobulin heavy-chain genes for every patient.

None of the samples from patients responding to antibiotic therapy contained t(11;18, q21;q21). This finding is in accordance with the report by Alpen and colleagues,[4] in which the translocation was not detected in 18 gastric MALT lymphomas responsive to *H pylori* therapy. By contrast, nine (75%) of the 12 antibiotic-resistant patients were positive for the translocation (table, figure), which suggests that the *API2-MLT* fusion protein might confer *H pylori*-independent survival, or growth, of gastric MALT lymphoma. Seven of the nine positive patients were at stage II_E or higher, which also suggests that t(11;18, q21;q21) is associated with more advanced stages of the disease.

Clearly, other genes might lead to a small number of t(11;18, q21;q21) gastric MALT lymphoma patients being non-responsive to *H pylori* eradication. Another cytogenetic abnormality, t(1;14, p22;q32), occurs infrequently in MALT-type lymphoma, and confers *H pylori*-independent growth of tumour cells. The translocation deregulates a novel gene, *BCL10*, which is proapoptotic. Mutations in *BCL10* result in loss of proapoptotic activity but gain of oncogenic potential, and have been found in three (27%) of 11 gastric MALT lymphomas that are resistant to *H pylori* eradication.[5] Whether t(11;18, q21;q21) and *BCL10* mutations are mutually exclusive in gastric MALT lymphomas non-responsive to *H pylori* therapy is unknown.

If confirmed on a large cohort of patients, detection of t(11;18, q21;q21) in gastric biopsies of MALT-type lymphoma will identify most cases that will not respond to antibiotic eradication of *H pylori*. This finding should be a great help to clinicians managing patients with non-responsive MALT-type lymphoma, and obviate the need for extended follow-up with repeated gastric endoscopy and biopsy. Detection of the translocation can be done in molecular biology laboratories, provided that fresh tissue has been retained. We are currently investigating methods for detection of t(11;18, q21;q21) in paraffin-embedded material in our laboratory.

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Effect of vancomycin and rifampicin on meticillin-resistant *Staphylococcus aureus* biofilms

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Decolonisation of patients with urinary catheter colonisation by meticillin-resistant *Staphylococcus aureus* (MRSA) is often difficult. Replacement of the catheter after prophylactic vancomycin administration has been one approach that is often unsuccessful in clinical practice. We suspected that formation of MRSA biofilms might account for the persistence of infection, and our study confirms this, also showing that MRSA is able to colonise a silastic rubber surface even in the presence of prophylactic vancomycin or rifampicin.

The increased use of implant devices such as lines, prostheses, and urinary catheters for care of patients brings with it an increased risk of infection. Such infections are frequently initiated by biofilms: surface-associated micro-organisms that are difficult to eradicate by chemotherapy.[1] Reports of biofilms and attempts to eradicate these