Persistent monoclonality after histological remission in gastric mucosa-associated lymphoid tissue lymphoma treated with chemotherapy and/or surgery: Influence of t(11;18)(q21;q21)

Keywords: MALT lymphoma, gastric lymphoma, H. pylori, t(11;18)(q21;q21)

Introduction

H. pylori eradication leads to histologic disappearance of gastric mucosa-associated lymphoid tissue (MALT) lymphoma in 60–90% of the patients with localised stage I disease [1–4], and most remissions are maintained for years [5–8]. Specific molecular features have crucial importance in the clinical behaviour of gastric MALT lymphoma, especially the presence of t(11;18)(q21;q21) which in most cases makes the lymphoma unresponsive to H. pylori eradication [9]. The response of gastric MALT lymphomas refractory to eradication, those with t(11;18)(q21;q21) and those with extensive disease, to other treatments is less clear. In localised or locally advanced disease, gastrectomy and/or radiotherapy can achieve responses in 80–90% of the patients [10–13]. In localised or advanced disease results with 45–100% of responses have been achieved with alkylating agents, [14] purine analogues such as 2CdA [15], combination chemotherapy [16] and anti-CD20 antibodies [17].

Despite the response of localised gastric MALT lymphoma to H. pylori eradication, 40–80% of the
patients in histologic remission have persistent monoclonal immunoglobulin heavy chain variable \((IgV_H)\) gene rearrangements for as long as 10 years [5–7]. There are no prospective studies to evaluate the molecular response of gastric MALT lymphoma treated with chemotherapy or local (radiotherapy or surgery) therapies and the scarce retrospective studies have contradictory results [18,19].

This retrospective study aimed to investigate the molecular response of patients with gastric MALT lymphoma achieving histologic remission after treatment with chemotherapy, surgery or a combination of both. Our results indicated that about 60% of the patients had persistent histologic persistence of the same monoclonal \(IgV_H\) rearrangement present at lymphoma diagnosis.

Materials and methods

Patients

The molecular response of 19 patients with gastric MALT lymphoma treated with chemotherapy and/or surgery who achieved a complete macroscopical and histological complete remission (CR) after treatment was studied retrospectively. Patients from two Hospitals in Spain (Hospital Ramón y Cajal in Madrid, and Hospital del Mar in Barcelona) were followed up for a minimum of 1 year after achieving histological remission. Only patients with a minimum of 1 year follow up after achieving histologic remission were acceptable for the study. Diagnosis of MALT lymphoma required the presence of characteristic infiltration by centrocyte-like lymphocytes and lymphoepithelial lesions [20]. Diagnosis was established by histological criteria only; monoclonal \(IgV_H\) gene rearrangement was not an obligatory criterion, as some cases showed polyclonal rearrangements [20]. All patients underwent a complete study of extension as detailed elsewhere [4] and were staged using the Lugano system [21]. Patients in all stages, I to IV were included. \(H.\ pylori\) status was determined in all cases with \(^{13}\)C Urea breath test and investigation of \(H.\ pylori\) in histological specimens and culture from gastric biopsies.

The patients had received a variety of initial treatments (diverse chemotherapy protocols, surgery or the combination of both) according to treatment policies followed at the time of diagnosis. \(H.\ pylori\) was eradicated in all patients when identified. CR was accepted after macroscopical and histologic disappearance of gastric disease in subsequent endoscopies and of all forms of extragastric disease, if previously documented. Re-staging was performed after treatment in all cases to assess the response and the clinical situation during follow-up. Sequential endoscopies and control of \(H.\ pylori\) status were performed throughout all these years, but without any fixed time schedule. Only patients achieving complete macroscopical and histologic remission after treatment were included in this study.

Histologic and molecular studies

The slides and paraffin blocks of the biopsies at diagnosis and from subsequent endoscopic controls during follow-up were retrieved from the archives of the Pathology Departments in the two participating hospitals. Histological and immunohistochemical studies were reviewed and reassessed. Molecular studies were performed in all available archival material.

PCR for the analysis of \(IgV_H\) gene rearrangements

Polymerase chain reaction (PCR) analysis of the \(IgV_H\) gene was performed for each patient using DNA extracted from two sets of five serial 5 \(\mu\)m sections cut from formalin-fixed paraffin-embedded tissue. In 13 cases, amplification of the \(IgV_H\) gene was performed using TaqGold polymerase and BIOMED-2 designed FR2 and FR3 primers for the \(IgV_H\) framework regions 2 and 3, and a consensus JH primer. PCR reactions were followed by heteroduplex analysis as recommended to avoid misinterpretation due to pseudoclonality [22], and amplification products were subsequently electrophoresed on polyacrylamide mini-gels, which were stained with ethidium bromide and viewed under UV light. In six other cases, FR1, FR2, FR3 and JH primers were used also according to the BIOMED-2 protocol and PCR products were analysed by capillary electrophoresis in an automated DNA sequencer (ABIPrism 3100, Applied Biosystem, Foster City, CA), as previously described [23]. All PCR reactions were performed in duplicate and appropriate positive and negative controls were used. The presence of 1 or 2 clearly dominant bands within the expected size indicated monoclonal rearrangements. For each patient, only reproducible bands of the same size through out all the samples were accepted as monoclonal to prevent spurious positivity.

Detection of \(t(11;18)(q21;q21)\). MALT1 rearrangements indicating the presence of \(t(11;18)(q21;q21)\) were analysed on diagnostic pre-treatment specimens. In 11 cases fluorescent \textit{in situ} hybridisation (FISH) with the commercially available break-apart LSI-MALT1 probe (Vysis, Downers Grove, IL) was used, as previously described [6]. In four other cases \(t(11;18)(q21;q21)\) was determined by reverse
transcriptase-PCR (RT-PCR) for API2/MALT1 fusion product in RNA obtained from the biopsy samples using primers that cover all the known breakpoints, as previously described [23].

**Sequencing of monoclonal PCR products.** PCR bands corresponding to monoclonal FR2/JH amplification products were directly excised from polyacrylamide gels and eluted in distilled water overnight. Sequencing was performed on both strands in an ABI PRISM 310 automatic sequencer (Applied Biosystem, Foster City, CA) using 10 μL of the eluted PCR band, FR2 and JH as sequencing primers, and the Big Dye terminator v1.1 cycle sequencing Kit (Applied Biosystem, Foster City, CA) following manufacturer’s procedures. The IgVH sequences obtained were compared with published germline sequences (VBASE sequence directory and Ig BLAST). The results obtained were confirmed by two independent experiments.

**Results**

**Clinical data**

The clinical features, treatment and outcome of the 19 patients are shown in Table I. Eleven patients had stage I, 5 stage II and 3 stage IV. *H. pylori* was identified in gastric biopsies (histology and/or culture) and C13 Urea breath test.

Table I. Clinical features, treatment, clonality and outcome.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Stage*</th>
<th>H. pylori†</th>
<th>t(11;18)</th>
<th>Treatment‡</th>
<th>Response§</th>
<th>PCR‡ baseline</th>
<th>PCR‡ follow-up</th>
<th>PCR: Last M** (months)</th>
<th>Outcome (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IV</td>
<td>+</td>
<td>−</td>
<td>CVP</td>
<td>NR</td>
<td>M</td>
<td>M</td>
<td>Mi</td>
<td>CR for 93 m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chlorambucil</td>
<td>PR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IV</td>
<td>−</td>
<td>+</td>
<td>CVP</td>
<td>NR</td>
<td>M</td>
<td>M</td>
<td>Mm</td>
<td>CR for 63 m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chlorambucil</td>
<td>NR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHOP</td>
<td>NR</td>
<td>M</td>
<td>M</td>
<td>Mm</td>
<td>CR for 13 m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cyclophosphamide</td>
<td>NR</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rituximab</td>
<td>NR</td>
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<tr>
<td></td>
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<td></td>
<td>Chlorambucil</td>
<td>CR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Surgery (TG)</td>
<td>CR</td>
<td>M</td>
<td>Pu</td>
<td></td>
<td>CR for 84 m.</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>−</td>
<td>ND</td>
<td>CVP</td>
<td>CR</td>
<td>NA</td>
<td>Mu</td>
<td>134</td>
<td>CR for 204 m.</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>CHOP</td>
<td>NR</td>
<td>M</td>
<td>Mm</td>
<td>13</td>
<td>CR for 13 m.</td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Chlorambucil</td>
<td>CR</td>
<td>M</td>
<td>Pm</td>
<td></td>
<td>CR for 30 m.</td>
</tr>
<tr>
<td>7</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>FCD</td>
<td>CR</td>
<td>M</td>
<td>Mm</td>
<td>9</td>
<td>CR for 15 m.</td>
</tr>
<tr>
<td>8</td>
<td>IV</td>
<td>+</td>
<td>−</td>
<td>Surgery (STG)</td>
<td>CR</td>
<td>M</td>
<td>Pu</td>
<td></td>
<td>Pleural relapse 13 m after surgery. CR for 132 m after CHOP. CR for 84 m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ CHOP</td>
<td>CR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Surgery (PG)</td>
<td>CR</td>
<td>M</td>
<td>Pu</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Chlorambucil</td>
<td>CR</td>
<td>M</td>
<td>Mi</td>
<td>63</td>
<td>CR for 84 m.</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Surgery (PG)</td>
<td>CR</td>
<td>M</td>
<td>Mm</td>
<td>41</td>
<td>CR for 107 m.</td>
</tr>
<tr>
<td>12</td>
<td>I</td>
<td>+</td>
<td>ND</td>
<td>Surgery (PG)</td>
<td>CR</td>
<td>NA</td>
<td>Pu</td>
<td></td>
<td>CR for 110 m.</td>
</tr>
<tr>
<td>13</td>
<td>I</td>
<td>+</td>
<td>−</td>
<td>Chlorambucil</td>
<td>CR</td>
<td>M</td>
<td>Mi</td>
<td>48</td>
<td>CR for 48 m.</td>
</tr>
<tr>
<td>14</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>Surgery (PG)</td>
<td>CR</td>
<td>M</td>
<td>Mm</td>
<td>60</td>
<td>CR for 60 m.</td>
</tr>
<tr>
<td>15</td>
<td>II</td>
<td>+</td>
<td>−</td>
<td>Chlorambucil</td>
<td>CR</td>
<td>P (FR3)</td>
<td>Mu</td>
<td>12</td>
<td>CR for 14 m.</td>
</tr>
<tr>
<td>16</td>
<td>I</td>
<td>+</td>
<td>ND</td>
<td>Surgery (TG)</td>
<td>CR</td>
<td>NA</td>
<td>Pu</td>
<td></td>
<td>CR for 98 m.</td>
</tr>
<tr>
<td>17</td>
<td>I</td>
<td>−</td>
<td>ND</td>
<td>Surgery (TG)</td>
<td>CR</td>
<td>NA</td>
<td>Pu</td>
<td></td>
<td>CR for 96 m.</td>
</tr>
<tr>
<td>18</td>
<td>II</td>
<td>+</td>
<td>−</td>
<td>Chlorambucil</td>
<td>CR</td>
<td>M</td>
<td>Mu</td>
<td>6</td>
<td>CR for 19 m.</td>
</tr>
<tr>
<td>19</td>
<td>II</td>
<td>+</td>
<td>−</td>
<td>Surgery (TG)</td>
<td>CR</td>
<td>M</td>
<td>Pm</td>
<td></td>
<td>CR for 48 m.</td>
</tr>
</tbody>
</table>

NA, not available; ND, not done.

*Stage: Lugano system [21].

†H. pylori: identified in gastric biopsies (histology and/or culture) and C13 Urea breath test.

‡Treatment: CVP: cyclophosphamide, vincristine, prednisone; FLU: fludarabine; CHOP: cyclophosphamide, vincristine, adriamycin, prednisone. FMD: fludarabine, mitoxantrone, dexametasone; FCD: fludarabine, cyclophosphamide, dexametasone; TG: total gastrectomy; PT: partial gastrectomy; STG: subtotal gastrectomy.


PCR: IgVH gene rearrangements: M, monoclonal; P, polyclonal; Mi, intermittent monoclonality pattern; Mm, maintained monoclonality pattern. Mu, monoclonality with uncertain pattern; Pm, maintained polyclonality; Pu, polyclonality with undetermined pattern. FR3, consensus primer for the VH region.

**PCR: Last M, time of the last M identified by PCR after CR.
identified in 15 of the 19 patients (79%) and successfully eradicated in all patients. Three patients (15.7%) had positive t(11;18)(q21;q21) and two of them had H. pylori colonization. Patients 5, 6, 10, and 13 received further treatment because of lack of lymphoma response to H. pylori eradication. The remainder were treated with chemotherapy and/or surgery due to locally advanced or disseminated disease or because they had been treated prior to 1993, before the "H. pylori era". Ten patients were treated with different chemotherapy regimens, chlorambucil alone, fludarabine or combination chemotherapy protocols. Three were treated with surgery alone and the remaining six with surgery (partial or total gastrectomy) followed by CHOP (cyclophosphamide, vincristine, adriamycin, prednisone) chemotherapy. All patients achieved CR that was maintained for a median of 63 months (IQR 30–98 months). Only one patient (patient 10) had a nodal relapse (without gastric relapse) 36 months after CR.

Clonality studies

Monoclonal IgVH gene rearrangements were found in the diagnostic biopsy of 14 patients. In the other five patients the initial biopsy was not available, not suitable for molecular study, or the study was incomplete, and therefore the baseline clonality could not be established (Table I and Figure 1).

During the follow-up, persistent monoclonal IgVH gene rearrangements were found in 11 of the 19 patients (58%); subsequently, we ascribed these cases to three different patterns of IgVH monoclonality: in five of them (26%) monoclonal rearrangements were present in all the samples examined during the study period (maintained monoclonal pattern), in three patients (15.7%) monoclonal IgVH gene rearrangements were detected intermittently (intermittent monoclonal pattern), and in the remaining three patients (15.7%) monoclonality was detected in the only control performed, but no pattern could be assigned as there was a single follow-up sample (undetermined monoclonality) (Table I and Figure 1). All three patients with t(11;18)(q21;q21) had a maintained monoclonal pattern. Polyclonal IgVH gene rearrangements were observed in eight patients (42%): a maintained polyclonal pattern was clearly established only in two patients, whereas in the remaining six patients it was not possible to assess the outcome of the clonal pattern as only one isolated study was performed; therefore, they were classified as having an undetermined polyclonal pattern.

Altogether, sequential data about IgVH clonality were available in 10 of 19 patients, and 8 out of these 10 patients with molecular follow-up (80%) had persistent monoclonality (five with maintained and three with intermittent IgVH monoclonal patterns). When found, IgVH monoclonality was detected for a median of 48 months (IQR 12–63) after the end of treatment and demonstration of CR. The persistence of monoclonal rearrangements did not condition the

![Figure 1. IgVH gene clonality at diagnosis and during follow-up. ◆ denotes monoclonal rearrangements and Δ denotes polyclonal rearrangements.]
relapse of the lymphoma in 18 of the 19 patients (97%). Only patient 10, who had a maintained intermittent monoclonal pattern during follow-up, had a nodal not a gastric relapse.

The diverse treatment modalities did not seem to affect the molecular response (Table I) and there was no significant difference in the occurrence of persistent IgVH monoclonal rearrangements in patients treated with chemotherapy or with chemotherapy and surgery when compared by means of a two-tailed Fisher exact test (data not shown).

**Discussion**

Present results showed that, as in the case of patients achieving CR after H. pylori eradication [5–7], most patients (58%) with gastric MALT lymphoma achieving CR after treatment with chemotherapy or surgery have clonal IgVH gene rearrangements for as long as 134 months after histological remission.

There are few previous studies on the same issue and their results are in some way controversial probably due to the different methods applied. In a series of 17 patients with localised gastric MALT lymphoma treated with involved field radiotherapy who achieved and maintained CR, Noy et al. [18] found that eight patients had persistent monoclonality and eight had intermittently monoclonal rearrangements. These data are comparable to those from this study. Alpen et al. [19], in a series of 20 patients with stage I–II gastric lymphoma in CR after treatment with CHOP chemotherapy with/without radiotherapy, demonstrated disappearance of monoclonality during follow-up. However, only four patients were MALT lymphomas; the remaining cases corresponded to diffuse large B-cell lymphomas (DLBCL). Taking into account only the gastric MALT lymphoma patients, their results could be comparable to ours since half of them had intermittent monoclonality during the follow-up.

An outstanding finding in our series is that patients bearing t(11;18)(q21;q21) in the original lymphoma showed during follow-up a persistent clonal rearrangement identical to the initial one for as long as 5 years after the histologic remission and this seems to indicate that these lymphomas did not achieve a true molecular remission. Although this finding was demonstrated in only three patients and should be consequently considered with some caution, it occurred in all t(11;18)(q21;q21) positive patients. Moreover, supporting of our results, persistence of molecular residual disease after CR has also been reported in other studies using a different approach such as the identification of translocation positive cells by demonstrating API2/MALT fusion product by RT-PCR. In one study t(11;18)(q21;q21) positive cells were detected in three patients treated with chemotherapy and/or Rituximab after 12 to 18 months in CR [23]. In another study [24], six patients also treated with chemotherapy or Rituximab achieved CR, but t(11;18)(q21;q21) positive cells were detected in gastric biopsies during follow-up: one patient relapsed and two others remained RT-PCR positive after 14 and 22 months. In the other three patients, RT-PCR was negative after 5, 7 and 29 months.

In contrast, sequencing analysis of the 2 t(11;18)(q21;q21) negative patients showed that follow-up clonal rearrangements were apparently unrelated to those of the initial lymphoma, suggesting that this group of lymphomas may have achieved a true molecular response after treatment, and that...
new unrelated clones may have emerged as a consequence of as yet unknown stimuli. The existence of receptor revision, as has been previously described in MALT lymphomas [25], can be ruled out in our patients because not only the VH segments but also the CDR3 regions were different in diagnostic and follow-up samples. Interestingly, the nodal relapse of patient 10 had the same clonal rearrangement that the initial gastric lymphoma, with no relation with those found during follow-up.

Most of the studies showing long-term persistence of B-cell monoclonality in gastric MALT lymphomas are based on the assumption that follow-up PCR bands of the same size as the original lymphoma clone correspond to identical rearrangements. However, the few studies in which sequencing analysis has been performed on sequential samples show that, at least in some cases, this is not the case. Wündisch et al. [7] reported 3 of 6 patients in CR after successful eradication treatment followed-up beyond 5 years, in which the final monoclonal PCR products were completely unrelated to the original lymphoma. Similarly, Thiede et al. [5] showed that in 2 of 3 patients in CR after eradication treatment who had monoclonality detected by PCR, microdissected basal lymphoid aggregates contained sequences not related to the initial lymphoma. Alpen et al. [19] reported the same IgVH allele in the diagnosis sample and during follow-up in three patients and completely different alleles in another patient. These latter results are not completely comparable, because most of these patients had DLBCL and molecular mechanisms and clonal responses may be different from those occurring in gastric MALT lymphoma [26,27]. Despite their limitations, the results of these studies seem to indicate that, at least in t(11;18)(q21;q21) negative cases, the eventual appearance of new clones may determine long-term IgVH clonal rearrangements in gastric MALT lymphoma patients in CR. In any of the three studies mentioned earlier the correlation between the sequencing results and the presence of t(11;18)(q21;q21) is given, so further studies are required to elucidate the possible association of t(11;18)(q21;q21) and molecular residual disease.

In the same way as with H. pylori eradication, in the group of patients treated with chemotherapy or surgery, the demonstration of ongoing monoclonality and even the persistence of the original lymphoma clone at molecular level, did not condition histological relapses in most lymphomas either t(11;18)(q21;q21) positive or negative. Persistent monoclonality alone is not an indication for further treatment unless a histologic relapse is found.

In conclusion, the long-term molecular follow-up of patients with gastric MALT lymphomas treated with chemotherapy and/or surgery showed that over half of them had persistent PCR monoclonal rearrangements despite prolonged histologic remissions. Both, the persistence of the initial lymphoma clone in t(11;18)(q21;q21) positive patients and the eventual appearance of new clones in negative patients may contribute to the occurrence of ongoing IgVH gene monoclonality.

Acknowledgements

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References


