A Polymorphism in the Complement Component C1qa Correlates with Prolonged Response Following Rituximab Therapy of Follicular Lymphoma

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Abstract

Purpose: Complement may play a role in the clinical response to rituximab and other monoclonal antibody–based therapies of cancer. The purpose of this study was to explore the relationship between the C1qa[276] polymorphism and the clinical response to rituximab in patients with follicular lymphoma.

Experimental Design: Genotyping for C1qa[276A/G] was done in 133 subjects with follicular lymphoma treated with single-agent rituximab, and correlation with clinical response was done using Cox regression analysis.

Results: Prolonged remission was observed among subjects that responded clinically to rituximab therapy and were carriers of the A allele compared with homozygous G subjects. Homozygous G subjects had a time to progression of 282 days, whereas A-allele carriers had a time to progression of 708 days [hazard ratio, (HR), 2.5; 95% confidence interval (95% CI), 2.0-3.1; P = 0.02]. Among subjects who achieved complete remission, homozygous G subjects had a time to progression of 250 days, whereas A-allele carriers had a time to progression of 1,118 days (HR, 4.5; 95% CI, 4.1-4.8, P = 0.04). The difference persisted after controlling for CD32 and CD16 polymorphisms. In patients who responded to rituximab used as first-line agent, a linear trend was observed among the C1qa[276] genotypes, with homozygous A subjects achieving complete response at a higher rate compared with heterozygous or homozygous G subjects.

Conclusions: Our findings indicate that polymorphisms in the C1qa gene may affect the clinical response and duration of response to rituximab therapy of follicular lymphoma. These results could have direct implications on designing antibodies with improved efficiency and enhance our understanding of the role of complement in monoclonal antibody therapy.

Rituximab, a chimeric anti-CD20 monoclonal antibody, has become a mainstay in the therapy of B-cell non–Hodgkin’s lymphoma since it was introduced in 1997. Used either alone or in combination with other agents, rituximab results in high response rates and some long-term remissions in patients with follicular lymphoma (1–4). The overall response rate in previously treated follicular lymphoma patients receiving rituximab monotherapy is 50% to 60%, including <20% of patients who achieve complete response. Responders have a median time to progression of 12 to 15 months (4, 5). Patients who relapse after a first therapy with rituximab may be retreated with comparable response rates and possibly an extended duration of remission (6, 7). When used as first-line therapy for follicular lymphoma, patients’ response rates are higher (73%), with about one third of all patients achieving complete response (1, 8, 9).

Despite its certain clinical value, the mechanisms responsible for the clinical antitumor effect of rituximab are not clear. The efficacy of anti-CD20 therapy with rituximab may be mediated through a combination of factors, which include complement activation (10–13). A number of groups, however, have found no evidence that complement is required for the antitumor effect of rituximab in C3- and C4-deficient rodent models (14, 15). Therapeutic activity of rituximab against murine cells expressing human CD20 was absent in syngeneic knockout mice lacking C1q, whereas depletion of natural killer cells, neutrophils, or the use of athymic nude mice did not affect the therapeutic activity of the drug (12). Nevertheless, in this xenograft model, the target cells express little of the complement-neutralizing molecules CD55 and CD59. Furthermore, the level of complement fixation or expression of the...
The induction of autoimmunity and the induction of an active antitumor response have some similarities (40–43). Because complement seems to be a critical modulator of the immune system’s tolerance to self-antigens (44), genetic polymorphisms that correlate with altered complement deficiencies and autoimmunity are logical targets for association studies in clinical outcome of cancer. Indeed, we observed in patients with breast cancer a significant correlation between C1QA gene polymorphisms and the rate of tumor dissemination (45). These data are consistent with the hypothesis that less active complement may result in enhanced cellular immunity and improved clinical outcome.

Taken together, these results suggest a number of hypotheses on how a C1QA polymorphism might have impact on response to rituximab therapy. Individuals with the C1QA[276A/G] allele could have lower levels of complement-mediated lysis, and therefore a poorer response rate to rituximab. Alternatively, impaired clearance of apoptotic tumor cells due to inefficient complement opsonization in individuals with C1QA[276A/G] allele could lead to improved cellular immunity against the lymphoma and therefore result in prolonged response to therapy. To begin assessing these hypotheses, we evaluated whether the C1QA[276A/G] polymorphism correlates with either response rate or duration in patients with follicular lymphoma treated with rituximab monotherapy.

Materials and Methods

Study population. The C1QA[276A/G] genotype was determined in 133 subjects with follicular lymphoma treated with rituximab monotherapy at the Stanford Medical Center, the Mayo Clinic, and the University of Iowa from 1993 to 2007. The 84 patients treated at the Stanford Medical Center represent the population previously explored for the impact of CD16 polymorphisms on response to rituximab (20). The 33 subjects treated at the Mayo Clinic were part of a study conducted in the North Central Cancer Treatment Group (8). The University of Iowa patients represented all follicular lymphoma patients treated with single-agent rituximab monotherapy for whom sera and detailed response and follow-up data were available. Sixty-two patients received rituximab as their first-line therapy. Seventy-one patients received rituximab for relapsed lymphoma after prior chemotherapy. A prerequisite for inclusion in this study was that the subjects did not receive chemotherapy within two months before initiation of the rituximab therapy and no maintenance therapy was administered. Patients received four weekly infusions of 375 mg/m² rituximab monotherapy using standard infusion guidelines. The study was conducted according to protocols approved by the institutional review boards at the University of Iowa, the Mayo Clinic, and Stanford University, and informed consent was obtained from all patients before blood sample collection.

Assessment of clinical response and duration of response to rituximab. Pretreatment staging consisted of physical examination; complete blood count; blood chemistry including lactic dehydrogenase; computed tomography scans of the chest, abdomen and pelvis; and bone marrow aspiration and biopsy. Follicular Lymphoma International Prognostic Index scores were not calculated as the preponderance of patients were treated at time of relapse. Rituximab response was determined by physical examination, computed tomography scanning, and pathology for bone marrow involvement if previously involved between 1 and 3 mo after last rituximab administration, and every 3 mo for the first year and every 6 mo thereafter until progression. The clinical response was then scored according to the International Workshop Response Criteria (46). The median length of follow-up after rituximab was 53 mo. Duration of response was defined as the...
time interval in days from the end of the rituximab therapy until progression of the disease or the last follow-up in patients who remained in partial or complete remission. Clinical management and assessment of disease status was done by individuals blinded to the C1q genotype.

**Specimen collection and processing.** Peripheral blood samples from all lymphoma patients were obtained before the initiation of rituximab therapy. Genomic DNA was purified using the PAXgene Blood DNA extraction kit according to the manufacturer’s instructions (Qiagen).

**Analysis of C1qA[276A/G] polymorphism.** Determination of the C1qA[276A/G] polymorphism was done blindly on coded specimens by restriction-fragment length polymorphism analysis as previously described (38). Briefly, the genomic DNA region containing the polymorphism was amplified by PCR and the amplicon purified with available commercial PCR product purification kits. Restriction-fragment length polymorphism analysis was done by enzymatic digestion with ApaI restriction endonuclease (New England Biolabs). Restriction digest fragments were separated in 2.5% agarose gels.

**Statistical analysis.** Subjects who had the 276 A/A or A/G genotype were designated as A carriers. Genotype and clinical response were compared using Cox regression analysis. The clinical responses of subjects with various C1qA[276] genotypes were compared using two-sided Fisher’s exact test. In addition to the C1qA[276A/G] polymorphism, the odds ratio of relapse after monoclonal antibody therapy associated with the C1qA[276] polymorphism. The C1qA[276A] polymorphism was done blindly on coded specimens by restriction-fragment length polymorphism analysis as previously described (38). Briefly, the genomic DNA region containing the polymorphism was amplified by PCR and the amplicon purified with ApaI restriction endonuclease (New England Biolabs). Restriction digest fragments were separated in 2.5% agarose gels.

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complete response was similar regardless of the C1qA_{276} genotype: 33%, 44%, and 36% for the A/A, A/G, and G/G genotypes, respectively.

Relapse rate after rituximab therapy correlates with C1qA_{276} genotype. During the first year after completion of rituximab therapy, subjects were evaluated at 3, 6, 9, and 12 months. At 3 months after completion of therapy patients were evaluated for best response and at later time points for relapse. The relapse rate results for the first follow-up year are summarized in Table 3. We observed that the rate of relapse was significantly higher in homozygous G subjects than in carriers of the A allele. In the first year of follow-up, 12 of 17 (71%) homozygous G patients relapsed, whereas only 21 of 73 (29%) of carriers of heterozygous and homozygous A subjects did so (P = 0.002). The correlation between the C1qA_{276} genotype and the relapse rates after rituximab was more evident among patients who achieved a complete response after rituximab. In this group, 4 of 5 (80%) homozygous G patients relapsed by the end of the first year, whereas only 6 of the 34 (18%) A-allele carriers did so. The rates of relapse were significantly higher for homozygous G patients at earlier time points as well: 40% versus 3% at 6 months and 60% versus 3% at 9 months for GG versus A carriers, respectively. No significant difference in the relapse rates has been observed between homozygous A and heterozygous subjects.

Duration of response to rituximab correlates with C1qA_{276} genotype. We next examined the relationship between the C1qA_{276A/G} polymorphism and the overall duration of rituximab response (Fig. 1). The A carrier who responded clinically to rituximab therapy had a median progression-free survival of 708 days versus 282 days for the homozygous G subjects [hazard ratio (HR), 2.5; 95% CI = 2.0-3.1; P = 0.026]. Among those subjects who achieved complete remission, the A carriers had time to progression of 1,118 days whereas G/G subjects had a time to progression of 250 days (HR, 4.5; 95% CI, 4.1-4.8; P = 0.046).

Multivariate analysis for C1qA_{276A/G}, FcγRIIIa (CD16) and FcγRIIa (CD32) polymorphisms. We used the Cox proportional hazards regression to evaluate whether the C1qA_{276} polymorphism (A carrier versus GG homozygous) is an independent prognostic factor from the CD16 (158 valine V or phenylalanine F) and CD32 (131 histidine H or arginine R) polymorphisms. Both CD16VV and CD32HH genotypes have been found to associate with higher affinity to human IgG and improved response rates and duration of response to rituximab therapy. When only the C1qA_{276} polymorphism is included in the Cox model, the A carrier significantly reduces the risk of relapse in responders by 50% (HR, 0.5; P = 0.026). When C1qA_{276} and either CD16 or CD32 polymorphisms are included in the model, the A allele still reduces the risk of relapse by 50%.

### Table 2. Response rate to up-front rituximab and rituximab as second-line agent

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Best response to rituximab</th>
<th>C1qA_{276} genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (%)</td>
<td>AG (%)</td>
</tr>
<tr>
<td>Up-front</td>
<td>Complete response (CR, CRu)</td>
<td>10 (53)</td>
</tr>
<tr>
<td></td>
<td>Partial response (PR)</td>
<td>5 (26)</td>
</tr>
<tr>
<td></td>
<td>Overall response</td>
<td>15 (79)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>19 (100)</td>
</tr>
<tr>
<td>Second-line</td>
<td>Complete response (CR, CRu)</td>
<td>4 (20)</td>
</tr>
<tr>
<td></td>
<td>Partial response (PR)</td>
<td>8 (40)</td>
</tr>
<tr>
<td></td>
<td>Overall response</td>
<td>12 (60)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Overall</td>
<td>Complete response (CR, CRu)</td>
<td>14 (36)</td>
</tr>
<tr>
<td></td>
<td>Partial response (PR)</td>
<td>13 (33)</td>
</tr>
<tr>
<td></td>
<td>Overall response</td>
<td>27 (69)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 (100)</td>
</tr>
</tbody>
</table>

Abbreviation: CR, complete response; CRu, complete response/unconfirmed.

### Table 3. Relapse rate after rituximab therapy according to C1qA_{276} genotype: first year follow-up

<table>
<thead>
<tr>
<th>Best response</th>
<th>All responders</th>
<th>P*</th>
<th>Complete responders</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval after mAb therapy (mo)</td>
<td>A carrier</td>
<td>GG</td>
<td>Relapsed</td>
<td>Ongoing %</td>
</tr>
<tr>
<td>1-3</td>
<td>0</td>
<td>73</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>65</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>59</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>52</td>
<td>29</td>
<td>12</td>
</tr>
</tbody>
</table>

Abbreviation: mAb, monoclonal antibody.

*Two-sided Fisher’s exact test, comparing homozygous G with A carrier (AA+AG). Percentages represent % relapsed from all patients under clinical evaluation at each time point.
The G allele of $C1qA_{276G}$, which is associated with shorter remissions to rituximab as outlined above, is also associated with higher C1q levels and measurements of complement-mediated lysis based on standard assays (38). This is opposite of what would be expected if the primary role of C1q was to participate in complement-mediated lysis of target cells.

There are a number of possible explanations for our findings. We now know that the effects of complement are much more complex. C1q has been reported to have a critical role in the phagocytosis of apoptotic bodies (27–30). In addition, there is evidence that there is an inverse relationship between C1q and antigen-presenting cell maturation and function (47). The increased incidence of lupus in both C1q-deficient patients and individuals lacking the $C1qA_{276G}$ allele provides further evidence of an inverse relationship between C1q activity and adaptive immunity (38, 39). Thus, one possible explanation for our findings is that the $C1qA_{276}$ G allele, with its more active complement, opsonizes tumor apoptotic bodies more effectively, thus facilitating their phagocytosis and removal. Rapid clearance of such material could limit the development of a cellular and humoral immune response, and hence results in a shorter progression-free interval. In contrast, in carriers of the A allele, with less complement function, the apoptotic bodies may be less efficiently opsonized by complement, thus providing more tumor cell fragments for dendritic cell–processing and presentation. If this is the case, the effect of the C1q polymorphism on tumor killing may go beyond antibody-based therapy of lymphoma. Indeed, we recently identified the $C1qA_{276}$ polymorphism as a significant indicator for improved metastasis-free survival in patients with breast carcinoma (45).

We recently reported that complement may actually interfere with rituximab-based killing. More specifically, we found that fixation of complement by rituximab-coated B cells limits natural killer cell activation and killing because the C3b component of complement interferes with the interaction between rituximab and the CD16 (48). If complement fixation actually limits the efficacy of rituximab, we would expect to observe a better response rate in patients with the $C1qA_{276}$ AA and AG genotypes, but it is less clear how this mechanism would explain the prolonged response.

Calreticulin, a natural receptor for C1q expressed by many cell types, has been reported to inhibit angiogenesis and tumor growth through its NH2-terminal region termed vasostatin (49). C1q interacts with calreticulin in the region responsible for the inhibitory effect of calreticulin on epithelial cell development and angiogenesis (50). Thus, C1q activity could affect the tumor microenvironment or angiogenesis with subsequent changes in duration of response.

In this population we have observed no association between the $C1qA_{276}$ polymorphism and the overall rate of response to rituximab therapy. Regardless of $C1qA_{276}$
genotype, roughly one third of subjects did not respond to monoclonal antibody treatment. A likely explanation for this observation is that the rate of initial response is primarily related to factors intrinsic to the malignant cell itself, such as surface expression of target molecules for monoclonal antibody therapy, and much less to host-innate immune characteristics, including complement. Nonetheless, duration of response relies on the efficient control of residual tumor growth that involves other components of the immune system, including antibody-dependent cellular cytotoxicity, natural killer cells, and cytotoxic T-cells.

We observed a trend for more patients with the C1qA[276] homozygous genotype to achieve a complete response to upfront rituximab therapy. This trend was not seen in patients who responded to anti-CD20 monoclonal antibody treatment after relapsing from or not responding to previous chemotherapy. In this later group, <25% of subjects achieved complete remission regardless of C1qA[276] genotype. This finding may be due to selection bias, as patients who relapse after chemotherapy are likely to have lymphomas with aggressive behavior that limits the efficacy of second-line rituximab therapy, therefore annulling any role C1q may have in the clinical outcome.

In conclusion, we have shown that a polymorphism in the C1qA[276] locus correlates with duration of rituximab response in follicular lymphoma patients. Although the differences between subjects in remission with different genotypes of C1qA are significant, we need to be cautious in the final interpretation of the results given the small size of the patient population that could be thus far be studied. Therefore, this study should be considered hypothesis-generating, not hypothesis-confirming. Ongoing studies are exploring the impact of this polymorphism in a larger population of lymphoma patients and in individuals with other cancers. In the laboratory, we are exploring the effect of C1q in general, and the C1qA[276] polymorphism in particular, on development of a cellular immune response. Confirmation of the clinical findings outlined above, and a better understanding of the mechanisms responsible, could have a major impact on prognosis and therapy for lymphoma and other malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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