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Chapter 3

Complement activation plays a key role in the side effects of rituximab treatment

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Abstract

Treatment with rituximab, a chimeric anti-CD20 monoclonal antibody, can be associated with moderate to severe first-dose side effects, notably in patients with high numbers of circulating tumor-cells. The aim of this study was to elucidate the mechanism of these side effects. At multiple early time points during the first infusion of rituximab, complement activation products (C3b/c and C4b/c) and cytokines (tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6) and IL-8) were measured in five relapsed low-grade non-Hodgkin’s lymphoma (NHL) patients. Infusion of rituximab induced rapid complement activation, preceding the release of TNF-α, IL-6 and IL-8. The level of complement activation was correlated both with the number of circulating B-cells prior to the infusion and with the severity of the side effects. We conclude that complement plays a pivotal role in the pathogenesis of side effects of rituximab treatment. Since complement activation can not be prevented by corticosteroids, it might be relevant to study the possible role of complement inhibitors during the first administration of rituximab.
Complement activation plays a key role in the side effects of rituximab treatment

Introduction

The chimeric anti-CD20 monoclonal antibody (mAb) IDEC-C2B8 (rituximab) has become an important treatment modality in low-grade non-Hodgkin's lymphoma (NHL)\(^1\)\(^2\) and its application in other CD20-positive B-cell malignancies (e.g. aggressive lymphoma's\(^3\)\(^4\), post-transplant lymphoma\(^5\)\(^6\), or chronic lymphocytic leukemia (CLL)\(^7\)\(^11\)), is rapidly expanding. In patients with low-grade follicular lymphoma and low numbers of circulating CD20-positive tumor-cells, treatment with rituximab was shown to be safe and well-tolerated.\(^1\)\(^2\) However, in patients with high numbers of circulating tumor-cells, rituximab treatment may be complicated by severe first-dose side effects, which can not be prevented by the usual prophylactic medication (i.e. paracetamol, antihistamine and/or corticosteroids).\(^7\)\(^12\)\(^13\) To get more insight into the pathogenesis of the infusion-related side effects of rituximab treatment, complement activation products (C3b/c and C4b/c) and cytokines (TNF-\(\alpha\), IL-6 and IL-8) were measured in serial samples obtained during the first infusion of rituximab in five low-grade NHL patients.

Patients and Methods

Patients

Relapsed low-grade NHL patients were treated in an ongoing study evaluating the safety and efficacy of the combination of rituximab (375 mg/m\(^2\) weekly x4) and granulocyte colony-stimulating factor (G-CSF) (filgrastim, Neupogen; 5 \(\mu\)g/kg/day, administered on three consecutive days starting two days before each infusion)(fig.1).\(^14\) Inclusion criteria for this study are: measurable progression of histologically confirmed CD20-positive B-cell lymphoma (Working Formulation A-C) after at least one and no more than three prior systemic therapies; expected survival of > 3 months; prestudy performance status of 0-2

Fig. 1 Treatment schedule

Patients were treated with the combination of rituximab and G-CSF. Rituximab was given weekly for 4 weeks. G-CSF (5 \(\mu\)g/kg/day) was administered for 3 days, starting 2 days before each infusion of rituximab (i.e., the third injection of G-CSF was administered several hours before rituximab infusion). Follow-up was at 1, 2 and 3 months.
according to the World Health Organization (WHO) scale; hemoglobin (Hb) > 5 mmol/L; white blood cell count (WBC) > 3.0 x 10^9/L; absolute granulocyte count > 1.5 x 10^9/L; platelet count (Pit) > 75 x 10^9/L; circulating tumor cells < 0.5 x 10^9/L; seronegative for human immunodeficiency virus (HIV) and hepatitis-B surface antigen (HBsAg); serum IgG > 600 mg/dL. The study was approved by the institutional ethics committee and performed according to the guidelines of the Declaration of Helsinki. All patients gave written informed consent before treatment was initiated. Rituximab was administered according to the guidelines described in the investigational drug brochure.\textsuperscript{15} Toxicity was evaluated according to the National Cancer Institute’s Adult’s Toxicity Criteria.

**Determination of complement activation and cytokine levels**

Patients received acetaminophen (1000 mg orally) and clemastin (2 mg intravenously) before onset of the infusion of rituximab as prophylactic medication. Corticosteroids were never given as premedication. Complement activation products and cytokine levels were measured before start of rituximab treatment (t=0) and at 30, 60, 90, 180 and 300 min after onset of the infusion. For measurement of complement activation, freshly drawn blood (5 mL) was collected in siliconized vacutainer tubes containing 0.1 mg/mL soybean trypsin inhibitor (SBTI), 10 mM EDTA and 20 mM benzamidine (final concentrations) to prevent any in vitro complement activation. Plasma was collected immediately by centrifugation and stored at -70 °C. Complement activation products (C3b/c and C4b/c) were measured by ELISA as described previously.\textsuperscript{16} Serum levels of IL-6, IL-8 and TNF-α were measured using a commercially available ELISA (Pelikan Compact ELISA kit, CLB, Amsterdam, The Netherlands), according to the manufacturer’s recommendations.

**Statistical analysis**

Correlations were determined using Pearson’s correlation coefficient. A P-value of <0.05 was considered significant.

**Results**

Complement activation products and cytokine levels were measured in 5 patients. Patient characteristics are listed in table 1. Side effects observed during the first infusion were fever and chills in 4 patients, in 2 patients requiring temporary discontinuation of the infusion and administration of corticosteroids (prednisone 25 mg intravenously) (table 2). One patient experienced dyspnea (grade 3) and flushes, starting 15 min after onset of the infusion. These side effects resolved after administration of corticosteroids, and infusion could be
Complement activation plays a key role in the side effects of rituximab treatment

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology (WF)</th>
<th>Sex/Age</th>
<th>Bulky Disease*</th>
<th>BM Involvement</th>
<th>Extranodal Disease</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>m/49</td>
<td>n</td>
<td>y</td>
<td>n</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>m/55</td>
<td>n</td>
<td>y</td>
<td>n</td>
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<tr>
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<td>B</td>
<td>m/75</td>
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<td>y</td>
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<td>E</td>
<td>B</td>
<td>f/28</td>
<td>n</td>
<td>y</td>
<td>n</td>
</tr>
</tbody>
</table>

*Bulky disease is defined as lesions > 7 cm. Abbreviations: WF = Working Formulation; BM = bone marrow

completed without further problems.

Levels of C3b/c, C4b/c and cytokines were within normal limits before start of the infusion of rituximab (fig. 2). An increase in both C3b/c and C4b/c was already observed 30 minutes after onset of the infusion. C3b/c reached a maximum at 180 min, while C4b/c reached a plateau at 90 min. Levels of TNF-α started to increase after 30 min, followed by IL-6 and IL-8. TNF-α reached a maximum at 60 min (177 ± 135 pg/ml (range 6-710)), IL-6 and IL-8 after 90 minutes (IL-6 298 ± 131 pg/ml; IL-8 150 ± 81 pg/ml).

The severity of the side effects observed in these patients, as reflected by both toxicity grade and the requirement to administer corticosteroids, was related to the number of circulating B-cells prior to the first infusion of rituximab. Furthermore, the maximum levels of C3b/c were correlated to the number of circulating B-cells prior to the infusion (r=0.85; P=0.07) (fig. 3). There was no correlation between the levels of the measured cytokines

Table 2 Clinical characteristics, complement and cytokine levels

<table>
<thead>
<tr>
<th>Pt</th>
<th>Circulating B-cells</th>
<th>PMN Side Effects</th>
<th>Toxicity Grade</th>
<th>Min After Start Infusion</th>
<th>Medication Required *</th>
<th>C3b/c</th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF-α</th>
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<tr>
<td>A</td>
<td>0.02</td>
<td>fever chills</td>
<td>1</td>
<td>60</td>
<td>n</td>
<td>130</td>
<td>231</td>
<td>34</td>
<td>40</td>
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<tr>
<td>B</td>
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<td>fever chills</td>
<td>2</td>
<td>50</td>
<td>y</td>
<td>483</td>
<td>613</td>
<td>366</td>
<td>131</td>
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<td>610</td>
<td>329</td>
<td>710</td>
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<tr>
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<td>n</td>
<td>175</td>
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<tr>
<td>E</td>
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<td>dyspnea flushes</td>
<td>3</td>
<td>15</td>
<td>y</td>
<td>1189</td>
<td>51</td>
<td>31</td>
<td>34</td>
</tr>
</tbody>
</table>

Circulating B-cells and neutrophils (PMN) (counts x10⁹/L) at start infusion, side effects and maximum levels of complement activation product C3b/c (nM) and cytokines (pg/mL) reached during the first administration of rituximab in 5 low-grade NHL-patients. *prednisone 25 mg intravenously
and the number of circulating B-cells.

Prior to the infusion, neutrophil counts had largely increased (table 2). This was not accompanied by an increase in cytokines or complement activation products (fig. 2). Furthermore, there was no correlation between the number of neutrophils before onset of the infusion and the maximum levels of cytokines or complement activation products during the infusion.

Discussion

In the present study, early complement activation, followed by cytokine release, during treatment with the chimeric anti-CD20 mAb rituximab are described. In agreement with other studies\textsuperscript{7,12,13}, we found that the severity of the side effects of rituximab treatment was dependent on the number of circulating B-cells prior to the infusion. Interestingly, although we only included patients with very low numbers of circulating B-cells (<0.51 x10\textsuperscript{9}/L), a correlation between the maximum levels of C3b/c and the number of circulating B-cells was observed. Such a correlation was not found for the levels of cytokines. This suggests that complement activation plays a key role in the pathogenesis of the side effects of rituximab treatment.

In line with or results, in a recent study complement activation was only found to occur in
patients with clinical side effects. Interestingly, complement activation was only observed in patients with bone marrow involvement. Unfortunately, in this study the number of peripheral blood B-cells was not mentioned. However, it is conceivable that those patients with bone marrow involvement have higher levels of circulating tumor-cells than patients without bone marrow involvement. This might explain the relation between complement activation and bone marrow involvement. In agreement with our study, no relation between IL-6 and complement activation or side effects was found.

Complement activation products are known to activate macrophages and mast cells, which are important sources of cytokines. Furthermore, complement activation products (e.g. C3a and C5a) can function as anaphylatoxins. We suggest that, during rituximab treatment, complement initiates cytokine release and is also directly responsible for some of the side effects (fig. 4). Thus, complement may be the central factor in the pathogenesis of the side effects of rituximab treatment.

![Diagram](image)

**Fig. 4**
Proposal for a mechanism explaining the pathogenesis of side effects occurring during treatment with rituximab.

Studies on the infusion-related side effects of OKT3 (a murine anti-CD3 mAb) support our hypothesis. During treatment with OKT3, a profile of complement activation and cytokine release similar to our findings with rituximab has been described. Interestingly, Buysmann et al showed that by administering OKT3 as a two-hour infusion in stead of an intravenous bolus injection, complement activation and side effects significantly decreased, whereas the cytokine release was not changed. Furthermore, Raasveld et al demonstrated complement activation without the release of cytokines in one OKT-3 treated patient, and
in this patient clinical side effects were comparable to the patients with increased cytokine levels.\(^{18}\) (and Raasveld, personal communication) Thus, these results in OKT3-treated patients support the importance of complement activation in the pathogenesis of side effects observed during treatment with monoclonal antibodies.

In a recent study in CLL-patients, treatment with rituximab was associated with severe side effects, which were described as a ‘cytokine-release syndrome’.\(^7\) However, in these CLL-patients, peak levels of cytokines were comparable to levels in our study patients, who had only mild side effects. This suggests that other factors, in addition to cytokines, may have played an important role in the pathogenesis of the side effects in these CLL-patients. Although not measured in the described study\(^7\), high levels of complement activation products may have been responsible for the severity of the side effects in these patients.

In the present study, patients were treated with the combination of rituximab and G-CSF. Importantly, although the three injections of G-CSF induced a significant increase in the number of neutrophils prior to onset of rituximab infusion, this was not accompanied by complement activation or increase in cytokines (fig. 2). Furthermore, side effects observed during the present study\(^14\) were similar to those reported in low-grade NHL-patients treated with rituximab monotherapy.\(^1\) Therefore, it is unlikely that the administration of G-CSF essentially altered complement activation and cytokine release during the infusion of rituximab. Moreover, we think it is justified to conclude that complement activation plays an important role in the pathogenesis of side effects of rituximab monotherapy as well.

In contrast to cytokine release, complement activation can not be prevented by corticosteroids.\(^{18,20,21}\) One possibility to decrease complement activation during mAb-infusion is by lowering the infusion rate of the antibody\(^{19,22}\), as has been applied to CLL-patients treated with rituximab.\(^7\) However, despite this strategy, first dose side effects during rituximab treatment can still be life threatening in patients with high numbers of circulating tumor-cells. Hence, it may be relevant to investigate the role of complement inhibitors\(^23\) during the first rituximab-infusion, especially in patients with high numbers of circulating tumor-cells, as they are at risk for severe first dose side effects.

Recently, an important role for complement-dependent cytotoxicity in the efficacy of rituximab was claimed, leading to the suggestion to enhance the efficacy of rituximab by enhancing complement lysis.\(^{24,25}\) However, other studies showed the importance of FcγR-mechanisms for the efficacy of rituximab.\(^{26}\) Thus, although the most important mechanism of action of rituximab in vivo has not been established yet, it is likely that not only safety, but also efficacy, is to some extent affected by complement. Therefore, studies investigating complement inhibitors in order to improve safety should carefully analyze the potential effects on clinical efficacy. Vice versa, studies exploring strategies to enhance complement activation should be aware of the possibility of increased toxicity.
Reference List


22. USA Package Insert, revised July 1999, IDEC Pharmaceuticals Corp. and Genentech Corp. USA Package insert. 2000