Brief report

Rituximab immunotherapy results in the induction of a lymphoma idiotypic-specific T-cell response in patients with follicular lymphoma: support for a “vaccinal effect” of rituximab

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The incorporation of rituximab, a chimeric anti-CD20 monoclonal antibody, into the therapeutic armamentarium for patients with follicular lymphoma (FL) has significantly improved treatment outcome for such patients. Despite the almost universal application of this therapy, however, its exact mechanism of action has not been completely defined. One proposed mechanism is that of a “vaccinal” effect, whereby FL cell kill by rituximab results in the elicitation of an FL-specific T-cell response. The demonstration that rituximab can even elicit such a response in patients has, to our knowledge, never been shown. We analyzed the response against the immunoglobulin expressed by the FL before and after rituximab monotherapy in 5 FL patients and found an increase in FL idiotype-specific T cells after rituximab in 4 of 5 patients. Our data thus provide “proof of principle” for the ability of passive immunotherapy with rituximab to elicit an active FL-specific cellular response. (Blood. 2009;113:3809-3812)

Introduction

Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant clinical activity in patients with follicular lymphoma (FL).1 The proposed mechanisms by which rituximab directly kills FL cells in vivo are complement-dependent cytolysis, antibody-dependent cellular cytotoxicity, or direct induction of apoptosis, although the contribution of each in mediating the clinical effects of rituximab is not well defined.2-10 In addition, there has been much recent speculation that rituximab has a “vaccinal effect,” eliciting an active immune response so to account for the ongoing clinical benefit often seen after a single treatment course.11 However, to date, there has been no evidence that rituximab treatment elicits such a lymphoma-specific T-cell response in patients with FL.

To determine whether rituximab treatment elicits a lymphoma-specific T-cell response, the frequency of lymphoma idiotype-specific T cells (Id-T cells) before (within several weeks of the first dose with no intervening therapy) and approximately 1 month after the last dose of rituximab was studied in 5 patients with FL.

Methods

Patients

Patients with FL treated with single-agent rituximab (375 mg/m2 weekly for 4 weeks) underwent blood drawing (patients 1 and 5), or leukapheresis (patients 2-4), as well as a lymph node biopsy under a University of Rochester Institutional Review Board–approved protocol. Informed consent was obtained in accordance with the Declaration of Helsinki.

Generation of idiotypic protein

Each idiotypic protein (Id) was generated from the node biopsy by Favville, Inc. (San Diego, CA) as previously described.12

In vitro stimulation and ELISpot

Monocyte-derived dendritic cells (DCs) were generated from prerituximab peripheral blood lymphocytes (PBLs) in Aim-V serum-free media, pulsed with autologous patient-derived Id protein, matured overnight, and then used to stimulate pretreatment or posttreatment lymphocytes for 1 week (patients 1 and 5) or 2 weeks (patients 2-4), as previously described.13-15 The resultant effector cells were rested overnight and then restimulated in interferon-γ (IFN-γ) ELISpot plates with mature DCs, DCs pulsed with either the patient’s lymphoma-specific Id (DC-Id), or an irrelevant Id (DC-irr; derived from a different patient), and the number of IFN-γ-secreting cells for each condition was determined according to the manufacturer’s recommendations (Mabtech, Mariemont, OH).

Statistical analysis

The primary endpoint was the number of IFN-γ-secreting T cells. The study design included 3 experimental conditions (DCs, DC-Id, DC-irr) and 2 time points (before and after rituximab). For each condition and time point, the primary endpoint was measured in triplicate. Two-way mixed analysis of variance models were used to describe the primary endpoint. This model included a random intercept for each patient as well as

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condition, time, and their interaction as factors. Hypothesis testing was conducted using likelihood ratio tests.

## Results and discussion

Patient characteristics are shown in Table 1. As shown in Figure 1A, for patients 1, 2, 3, and 5, there is an increase in the number of IFN-γ–secreting cells on restimulation of 1 week (patients 1 and 5) or 2 weeks (patients 2 and 3) in vitro stimulation (IVS) effector cells with DC-Id compared with that seen with either DCs or DC-irr in the postrituximab-treated PBLs. This was not the case for patient 4 at 2 weeks, however. In contrast, the number of IFN-γ–secreting cells from the prerituximab PBLs showed either no or minimal Id–T-cell responses in all 5 patients. Enough cells were available to repeat the studies for patients 2, 3 and 4, and similar results were obtained (data not shown). As noted in Table 1, patient 2 received a granulocyte-macrophage colony-stimulating factor–idiotype-keyhole limpet hemocyanin vaccine (using the “vac-Id”) before this study. At relapse, a new dominant clone emerged, using a different Id (Id‘) than that used for the Id vaccine, emerged. This new Id (Id‘) was used in the current study.

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Pathology</th>
<th>Prior treatment</th>
<th>Rituximab response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Follicular, grade 2</td>
<td>None</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>Follicular, grade 1</td>
<td>Rituximab, GM-CSF–Id-KLH vaccine*</td>
<td>PR</td>
</tr>
<tr>
<td>3</td>
<td>Follicular, grade 3</td>
<td>DLBCL treated with R-CHOP at 6 cycles and APBSCT, relapsed with follicular, grade 3, treated with IFXRT</td>
<td>PD</td>
</tr>
<tr>
<td>4</td>
<td>Follicular, grade 1</td>
<td>None</td>
<td>PR</td>
</tr>
<tr>
<td>5</td>
<td>Follicular, grade 1</td>
<td>None</td>
<td>PR</td>
</tr>
</tbody>
</table>

GM-CSF indicates granulocyte-macrophage colony-stimulating factor; Id-KLH, idiotype–keyhole limpet hemocyanin; DLBCL, diffuse large B-cell lymphoma; R-CHOP, rituximab, cyclophosphamide, adriamycin, vincristine, prednisone; APBSCT, autologous peripheral blood stem cell transplantation; IFXRT, involved field radiation therapy; CR, complete response; PR, partial response; and PD, progressive disease.

*At the time of relapse and rituximab retreatment, a new dominant clone, using a different Id (Id’) than that used for the Id vaccine, emerged. This new Id (Id‘) was used in the current study.

If rituximab elicits a lymphoma-specific Id–T-cell response, then (1) the number of IFN-γ–secreting cells after stimulation with DC-Id would be greater than DCs in the postrituximab samples compared with the prerituximab samples; therefore, the difference in the number of IFN-γ–secreting cells between DC-Id and DCs in the postrituximab samples minus the difference in the number of IFN-γ–secreting cells between DC-Id and DCs in the prerituximab samples (the difference of the difference) would be greater than zero; (2) DC-Id would also be greater than DC-irr in the postrituximab samples compared with the prerituximab samples and would therefore also be greater than zero, whereas (3) DCs and DC-irr in both the pre- and postrituximab samples would be the same and thus would be centered around zero. In Figure 1B, data show the difference between the numbers of IFN-γ–secreting cells stimulated by DCs and DC-irr; DC-Id and DC; and DC-Id and DC-irr, before and after rituximab for each patient. The data from patients 1, 2, 3, and 5 meet the aforementioned criteria, whereas the data from patient 4 do not (Figure 1B symbols). Data from all 5 patients were pooled (displayed as bars in Figure 1B), and a 2-way mixed analysis of variance, accounting for intrapatient correlation, was used to assess statistical significance.16 Using this analysis, we show that there was a significantly greater response elicited by DC-Id, compared with that of DCs, in the postrituximab samples, also compared with the prerituximab samples ($P = .017$). In addition, there was a significantly greater response elicited by DC-Id, compared with that of DC-irr, in the postrituximab sample, also compared with the prerituximab samples ($P = .016$). In contrast, there was no statistically significant difference between the responses to DCs versus DC-irr comparing the pre- and postrituximab samples ($P = .92$). To confirm that the conclusions were not complicated by the prior Id vaccination of patient 2, the statistical analysis was performed excluding this patient, and the results remained significant ($P < .05$). Taken together, these data provide proof of principle that rituximab can elicit an Id–T-cell response in patients with FL.

The rationale as to why rituximab might elicit an active cellular immune response is that, regardless of its mechanism of action, rituximab treatment probably results in the inflammatory death of lymphoma cells. This would result in a depot of tumor antigens released in the context of a microenvironment favoring the uptake, processing, and presentation of tumor-associated antigens by DCs to T cells, resulting in the elicitation of a cell-mediated lymphoma-specific immune response.17-19 Finally, the engagement of DCs activating FcγRs by rituximab may further induce DC activation and maturation, thus favoring the generation of a cellular immune response.20,21

In our study, there was no obvious association between the clinical response to rituximab and the elicitation of an Id–T-cell response. This is not surprising because there are probably multiple interacting mechanisms that determine clinical response. In addition, rituximab probably also elicits T-cell responses to lymphoma-specific antigens other than the idiotype, which were not captured in this study. Our study does show, for the first time, however, that passive immunotherapy with rituximab results in the induction of an active cellular response directed against a lymphoma-associated antigen. To determine whether this is indeed a bona fide mechanism of action of rituximab will require an analysis of a larger number of patients as well as the correlation of immune responses to clinical responses.

The demonstration that rituximab generates an active lymphoma-specific immune response could have profound implications as to how we optimally treat patients with FL. Specifically, if the elicitation of a lymphoma Id–T-cell response having immunologic
Figure 1. Rituximab treatment elicits a lymphoma idiotype–specific IFN-γ T-cell response. Immature DCs from all 5 patients were generated, pulsed with autologous patient lymphoma-derived idiotype protein (Id), and matured overnight. Pulsed/matured DCs were then used to stimulate pre- or postrituximab treatment lymphocytes for 1 week (patients 1 and 5), or 2 weeks (patients 2-4) in Aim-V serum-free media with interleukin-2 (IL-2). The resultant week 1 or 2 effectors were then harvested, washed, and rested overnight in Aim-V media containing IL-2. Rested effectors were stimulated in IFN-γ ELSpot plates (in triplicate) with mature DCs alone or DCs pulsed and matured with either the patient’s lymphoma-specific Id (DC-Id) or an irrelevant Id derived from a different patient (DC-Irr). The number of IFN-γ-secreting cells for each condition was then determined by standard ELSpot methods after incubation overnight. (A) The number of IFN-γ spots for patients 1 through 5, for both pre- or postrituximab effectors stimulated overnight by (DC), (DC-Id), (DC-Irr) are shown, as is the IVS repeat for patient 2 using the vaccine-specific Id for the IVS rather than the relapse Id. (B) The difference between the numbers of IFN-γ-secreting cells on stimulation with (DC) DCs versus DC-Irr (DC-Id versus DC-Irr) was calculated for each of the pre- and postrituximab samples (eg, DC-Id − DCIrr), and then the difference between the pre- and postrituximab samples was calculated (eg, [DC-Id − DCIrr]pre − [DC-Id − DCIrr]post). A final difference that is greater in the postrituximab sample, compared with the pretriximab sample, is plotted as a positive number (more than zero), whereas a difference that is similar between the pre- and postrituximab samples is plotted around zero. The data are shown for each individual patient (symbols), as well as for the averages computed over all 5 patients for each condition (bars), with the corresponding P values from tests assessing the significance of each bar.
memory can be augmented, it is possible that both the progression-free and overall survival after rituximab may be prolonged. Given that the Id-specific responses shown in this study were not very robust, to elicit such clinically relevant responses, rituximab treatment will need to be coupled with strategies designed to overcome the FL-induced inhibition of the immune response. Consistent with this assumption, patient 5 had the most robust response and a very low tumor burden, which is typically thought to be associated with less tumor-associated immune suppression. We and others have shown that one such mechanism of FL-associated immune suppression is increased numbers of Tregs within the FL microenvironment.22,23 The challenge will be to overcome these and other mechanisms of tumor-induced immune suppression.24

Acknowledgments

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References


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Authorship

Contribution: S.P.H. designed and performed research, analyzed data, and wrote the paper; O.H. performed statistical analysis and wrote the paper; and T.R.M., A.M.L., J.W.F., F.Y., R.I.F., R.J.K., R.B.B., and S.H.B. designed research, analyzed data, and wrote the paper.

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