Phase I Trial of a Novel Intradermal Idiotype Vaccine in Patients with Advanced B-Cell Lymphoma: Specific Immune Responses Despite Provocative Immunosuppression

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Abstract

The immunoglobulin receptor of B-cell lymphomas constitutes a specific tumor antigen (idiotype) and a target for active immunotherapy. Encouraging results have been reported in phase II trials after s.c. vaccination of follicular lymphoma patients during clinical remission with idiotype produced from eukaryotic cell lines and coupled to an immunogenic carrier macromolecule. We have developed a good manufacturing protocol for rapid expression of idiotype vaccines as recombinant Fab fragments in Escherichia coli. The objectives of this trial were to show safety and feasibility of intradermal immunization with this vaccine and to investigate whether immune responses were induced by this immunization route. Patients (n = 18) with advanced B-cell malignancies received repetitive intradermal vaccinations with 0.5 to 1.65 mg recombinant idiotype Fab fragment mixed with lipid-based adjuvant in combination with 150 μg granulocyte macrophage colony-stimulating factor s.c. at the same location. The patients’ immune status was assessed by flow cytometry of peripheral blood lymphocytes and concomitant hepatitis B vaccination. Cellular and humoral immune responses to the vaccine were assessed by enzyme-linked immunospot and ELISA. Side effects of a total of 65 vaccinations were mild and did not affect the immunization schedule. No patient developed hepatitis B surface antibodies (anti-HBs) after two hepatitis B immunizations. Of 17 evaluable patients, five developed hepatitis B surface antibodies (anti-HBs) after two hepatitis B immunizations. Of 17 evaluable patients, five developed anti-HBs. Stimulation of peripheral blood T cells from lymphoma patients during clinical remission (8–11). Induction of immunity was associated with superior outcome (9, 12). Some objective tumor regressions upon idiotype vaccination have also been reported (8, 13, 14).

With the exception of multiple myeloma and Waldenstrom’s macroglobulinemia, where soluble idiotype protein may be purified from the patient’s serum (15, 16), idiotype vaccines from non-secreting lymphomas must be manufactured. Viable lymphoma cells from a biopsy may be fused with a myeloma cell line by somatic cell hybridization followed by purification of idiotype from the culture supernatant of the resulting heterohybridoma (17). Several strategies for production of recombinant idiotype protein in eukaryotic cells with an appropriate expression system have been proposed (i.e., expression as an IgG3 in a murine lymphoma cell line, as IgG1 in sf9 insect cells, and as scFv fragment in tobacco plants; ref. 18). In almost all reported clinical trials with these vaccines, the weakly immunogenic idiotype protein was covalently coupled to the potent immunogenic carrier molecule keyhole limpet hemocyanin (KLH).

To ease manufacturing of individual idiotype vaccines and to shorten production times, we have adapted a bacterial expression system for recombinant immunoglobulin Fab fragments to the production of recombinant idiotype vaccines in Escherichia coli (19, 20). Stimulation of peripheral blood T cells from lymphoma patients with such recombinant, lymphoma-derived Fab fragment resulted in specific, MHC class I–restricted cytotoxic activity against autologous, idiotype-expressing target cells (21). Because coupling rates to KLH were too variable to fulfill good manufacturing (GMP) criteria,3 we decided to test alternative strategies to obtain high immunogenicity without further chemical vaccine modification. Intradermal vaccination with a reduced antigen dose has recently been shown to be equipotent to a conventional s.c. influenza vaccine, presumably due to antigen delivery to professional antigen-presenting cells (APC) of the skin (22). In addition, novel lipid-based adjuvants with superior immunogenicity, such as MF59, have been introduced into commercially available vaccines (23). Based on these advances in the design of vaccines against common infectious agents, we chose to apply a mixture of the recombinant idiotype with MF59 via an intradermal injection route. Because this trial tested to our knowledge for the first time a drug manufactured individually in E. coli, and because significant toxicities of the intradermal anti-lymphoma immunity in animal models (1–4). Depending on the tumor model and the vaccine formulation, both cellular and humoral immunity may play a role in tumor rejection (5–7).

Idiotype-specific immune responses and disappearance of minimal residual disease (MRD) have been observed after idiotype immunization in follicular lymphoma patients during clinical remission (8–11). Induction of immunity was associated with superior outcome (9, 12). Some objective tumor regressions upon idiotype vaccination have also been reported (8, 13, 14).

Introduction

B-cell non-Hodgkin’s lymphomas (B-NHL) are clonal proliferations of cells expressing an identical antigen receptor. The hyper-variable parts of this immunoglobulin form a unique idiotype of the malignant clone and represent a tumor-specific antigen. Immune responses to the lymphoma idiotype induces protective and therapeutic anti-lymphoma immunity in animal models (1–4). Depending on the tumor model and the vaccine formulation, both cellular and humoral immunity may play a role in tumor rejection (5–7).

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3F. Simon, unpublished observations.


Research Article
Lymphoma-derived variable immunoglobulin segments were inserted into of the idiotype heavy (H) and light (L) chain expressed by the lymphoma. GMP-Grade individual idiotype vaccines were produced commercially (CellGenix (www.aacrjournals.org 4497 CancerRes2006;66:(8).April15,2006). All clinical procedures were reviewed and approved by the institutional review board of Humboldt University Medical School, Freiburg, Germany. They were performed according to the Declaration of Helsinki. In a typical phase I design, patients with B-cell malignancies in advanced stages that had relapsed or progressed after a full treatment course with anthracyclin- or fludarabine-containing chemotherapy, autologous hematopoietic stem cell transplantation, or progressive disease; PR, partial remission; NS, not significant; ND, not done; NA, not applicable due to early disease progression; RF, rheumatoid factor.

### Patients and Methods

#### Subject eligibility.
In a typical phase I design, patients with B-cell malignancies in advanced stages that had relapsed or progressed after a full treatment course with anthracyclin-or fludarabine-containing-containing or high-dose chemotherapy with autologous stem cell transplantation were enrolled after giving informed consent. Patients were at least 18 years old and had a life expectancy of at least 6 months. Exclusion criteria were severe concomitant infections or infections, active central nervous system involvement, and immunosuppressive or antineoplastic therapy (including irradiation) 4 weeks before vaccination. The trial was approved by the Institutional Review Board and was conducted according to the Declaration of Helsinki.

#### Vaccine production.
Lymphoma tissue was obtained by lymph node biopsy (n = 9), bone marrow aspiration (n = 7), or phlebotomy (n = 2). GMP-grade individual idiotype vaccines were produced commercially (CellGenix GmbH, Freiburg, Germany) by anchored reverse transcription-PCR cloning of the idiotype heavy (H) and light (L) chain expressed by the lymphoma. Lymphoma-derived variable immunoglobulin segments were inserted into pFab-H or pFab-L, and recombinant idiotype protein was expressed in E. coli as a recombinant IgG1 Fab fragment (20). Fab protein was purified by affinity chromatography and gel filtration. The success rate for Fab production is currently 89% in a continued series of 78 consecutive vaccine productions with a median purity of 99% heterodimeric Fab protein (range, 72-100%) and a median yield of 17.0 mg (range, 1.2-250 mg) from a single 14-liter fermentation run.4

### Treatment schedule.
One vaccination consisted of an intradermal injection of 10 μg/μL idiotype solution mixed 5:1 (v/v) with MF59 adjuvant (Chiron Behring, Marburg, Germany; ref. 23). The minimum dose of idiotype protein was 0.5 mg per vaccination and was planned to be increased according to the modified Fibonacci design after treatment of three patients at each dose level without relevant side effects. In addition, 150 μg granulocyte macrophage colony-stimulating factor (GM-CSF; molgramostim; Leucome; Novartis, Nuernberg, Germany) were injected s.c. at the same anatomic location. Vaccinations were given in weeks 0, 3, 5, and 9.

Beginning with the fourth patient enrolled, anti-HBs-negative patients received a standard hepatitis B vaccine (Gen-HB-Vax; Aventis Pasteur, Frankfurt, Germany) at the first and third idiotype vaccination. Staging according to National Cancer Institute criteria (24) with bidimensional measurement of lymphomatous masses or by quantitative assessment of appropriate disease variables [e.g., chronic lymphocytic leukemia (CLL), lymphocyte counts; myeloma, immunoglobulin concentration] was done within 1 week before the first vaccination, at the third vaccination, 2 weeks after the fourth vaccination, and at weeks 16, 24, and 52. Disease progression at any time excluded the patient from further follow-up. Side effects were graded according to WHO criteria (25). Blood samples for routine clinical chemistry, quantitation of lymphocyte subsets by dual-color flow cytometry (antibodies from BD Biosciences, Heidelberg, Germany), assessment of cellular and humoral immunity against idiotype, and screening for autoantibodies were taken on the days of the staging examinations.

### Detection of idiotype antibodies by ELISA.
Microtiter plates (Nunc, Wiesbaden, Germany) were coated with 10 μg/mL recombinant Fab

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> Details to be published by Bertinetti et al., submitted.
fragment in 50 mmol/L sodium carbonate buffer (pH 9.6) and blocked with 0.1 mol/L Tris-HCl (pH 7.6)/0.05% Tween 20. Serially diluted sera were added for 1 hour at room temperature. Horseradish peroxidase (HRP)–labeled goat F(ab′)2 anti-human IgM (Biosource, Camarillo, CA), anti-human IgG-Fc (Calbiochem, La Jolla, CA), or biotin-conjugated mouse anti-human κ or λ (BD Biosciences) served as secondary antibodies. After additional incubation with Streptavidin-HRP (BD Biosciences) for biotin-conjugated secondary antibodies, bound HRP was detected with ABTS solution (Roche, Mannheim, Germany) and absorbance measurement at 405 nm. According to previously described criteria (12), induction of an idiotype-specific in vivo antibody response was defined as a 4-fold titer increase compared with pre-vaccination serum and to a control Fab.

**Generation of idiotype-presenting monocyte-derived dendritic cells.** Peripheral blood mononuclear cells (PBMC) were adhered to culture flasks for 2 hours at 37°C in RPMI (Life Technologies/Invitrogen, Karlsruhe, Germany) supplemented with 0.5% HSA (Baxter Germany, Munich, Germany) followed by culturing in serum-free medium (CellGro DC; CellGenix) with 100 ng/mL GM-CSF (R&D Systems, Wiesbaden-Nordenstadt, Germany) and 50 ng/mL interleukin-4 (IL-4; Chiron, Ratingen, Germany) for 1 week. Only experiments with 200 to 300 spots per well in the pokeweed mitogen controls and with more than three background spots (without antigen) per 10^5 cells were analyzed. A T-cell response was defined as a significant (P < 0.05) increase of the response to the vaccine idiotype by two-tailed t test. The specificity of T-cell responses was analyzed with patient and control idiotype as ELISPOT targets by a Poisson regression model accounting for repeated measurements using the generalized estimation equations method for variable estimation with SAS version 8 software (SAS Institute, Cary, NC; ref. 26). This model includes the different conditions under which measurements were taken as independent variables. The estimated effects in this model then represent the ratios that relate average counts under one condition to another. All other statistical calculations were done with GraphPad Prism software (GraphPad Software, San Diego, CA).

### Results

**Treatment administration and side effects.** Eighteen patients (13 male and 5 female) with various B-cell malignancies and a median age of 53.5 years (range, 32-75 years) were enrolled in the trial. The dose was 0.5 mg idiotype protein per vaccination in 13 patients, 1.0 mg in three patients, and 1.65 mg in two patients. Further dose escalation was abandoned because the accumulating production experience suggested that it would be unlikely to obtain sufficient vaccine without repeated fermentation runs. Localized erythema, hyperthermia, and transient skin induration of up to 12 cm, which tended to increase with each vaccination, developed at the vaccination site but resolved after a few days. Grade 3 anemia and thrombocytopenia developed in one case each who were already in grade 2 cytopenia immediately before vaccination. All other symptoms and side effects were mild (Fig. 1), and all vaccinations were given according to schedule.

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**Table 1. Patient characteristics, vaccinations, immunologic responses, and time to progression (Cont’d)**

<table>
<thead>
<tr>
<th>No. vaccinations</th>
<th>Vaccine dose (mg)</th>
<th>T-cell response by t test</th>
<th>Antibody response, titer increase</th>
<th>Auto-antibody</th>
<th>Time to progression (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>P = 0.032</td>
<td>Negative</td>
<td>Trace ANA</td>
<td>≥5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>—</td>
<td>NA</td>
</tr>
<tr>
<td>4 (+4)</td>
<td>1.0</td>
<td>P &lt; 0.0001</td>
<td>IgM 8×; IgG 64×</td>
<td>RF = 17 IU/l</td>
<td>5</td>
</tr>
<tr>
<td>4 (+4)</td>
<td>1.0</td>
<td>P = 0.052</td>
<td>IgM 128×; IgG 128×</td>
<td>Trace ANA</td>
<td>9</td>
</tr>
<tr>
<td>4 (+4)</td>
<td>1.0</td>
<td>(P = 0.052)</td>
<td>IgM 6×; IgG 8×</td>
<td>RF = 37 IU/l</td>
<td>≥36</td>
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<tr>
<td>3</td>
<td>1.65</td>
<td></td>
<td>IgM 8×; IgG 2×</td>
<td>Negative</td>
<td>≥27</td>
</tr>
<tr>
<td>4 (+2)</td>
<td>1.65</td>
<td></td>
<td>IgM &lt;2×</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>4 (+3)</td>
<td>0.5</td>
<td></td>
<td>IgM &lt;2×; IgG 6×</td>
<td>(ANA, RF)</td>
<td>≥39</td>
</tr>
<tr>
<td>4 (+4)</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>P &lt; 0.0001</td>
<td>Trace ANA</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4 (+3)</td>
<td>0.5</td>
<td></td>
<td>IgM 2×; IgG 32×</td>
<td>—</td>
<td>≥27</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>P = 0.0079</td>
<td>Negative</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>NS</td>
<td>Negative</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

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http://www.syfpeithi.de.
In six patients, testing for autoantibodies indicated possible subclinical autoimmune activity 11 to 24 weeks after start of idiotype vaccination (Table 1). One additional patient (A21) with follicular lymphoma and rheumatoid arthritis had an approximate doubling of rheumatoid factor to 586 IU/mL at 24 weeks after the first idiotype vaccination but no clinical exacerbation of his arthritis at the metacarpophalangeal joints.

T-cell status and cellular immune responses. Patients without circulating lymphoma cells had low normal to severely depressed circulating CD4+ T-cell counts before vaccination. Prior treatment with fludarabine was associated with a particularly poor T-helper cell status but had a lesser effect on cytotoxic T cells (Fig. 2A). In accordance with published data (27, 28), the CLL/lymphocytic lymphoma patients had normal to high CD4+ and CD8+ T-cell counts even when pretreated with fludarabine, but these cells are known to be functionally incompetent (29). Induction of T-cell-mediated immunity to the vaccine was detected by ELISPOT in eight patients (Fig. 2B; Table 1). Induction of T-cell responses seemed to be independent of T-helper cell counts before vaccination (Fig. 2C). Because four of these patients also had an increase of T-cell reactivity to a randomly chosen control Fab (Fig. 2B), we did a combined Poisson regression analysis of the ELISPOT results. This analysis proved predominant specificity of the T-cell response for the vaccine compared with randomly chosen control Fabs (Fig. 2D).

A comparison of ELISPOT data of patient A26 with two different control Fabs provided further insight into the molecular basis for a specific anti-idiotype T-cell response (Fig. 3). The T-cell response induced by the vaccination was stronger to the vaccine Fab than to a control Fab with low V\textsubscript{H} sequence homology (A14). In contrast, a highly homologous control Fab (A21) with the same V\textsubscript{H} gene as the vaccine Fab contained numerous shared potential epitopes as revealed by epitope prediction algorithms. Accordingly, no preferential T-cell reactivity to the vaccine could be detected.

Availability of sufficient T cells permitted a formal epitope mapping in patient A83 (Fig. 4). T-cell epitopes derived from various positions in the idiotype H chain and predicted to be presented by the patient's HLA haplotype by reverse immunology were tested separately and compared with a peptide from a control Fab with similar HLA-binding scores. Recognition of C region peptides was significantly inferior to the control peptide. In contrast, a significantly higher response was detected against a peptide from the CDR2 region (an HLA-restricted CDR3 peptide could not be identified in this case).

B-cell status and humoral immune responses. Pretreatment effects on the B-cell compartment were less pronounced than on T cells: nonleukemic patients who had received rituximab before vaccination did not have significantly less B cells than rituximab-naive patients (Fig. 5A). None of 10 patients who were evaluable for anti-HBs responses after two hepatitis B vaccinations developed any detectable anti-HBs antibodies. This subgroup did not differ from the remaining patients with respect to fludarabine (P = 0.10, Fisher’s exact test) or rituximab pretreatment (P = 0.64), CD4+ T-cell counts (P = 0.7, two-sided Mann-Whitney test), or B-cell counts (P = 0.6).

A specific increase of anti-idiotype antibodies was detected in five patients (Table 1). These humoral responses were confirmed in all cases with anti-‘‘opposite’’ L chain detection antibodies (i.e., anti-\lambda detection antibody for idiotypes with a \kappa Ig chain and vice versa; data not shown). Two additional patients developed a humoral anti-Fab response with substantial cross-reactivity to different human Fabs but not to a murine control Fab (Table 1). Humoral immune responses were not correlated to T-helper or B-cell counts (Fig. 5B). Anti-idiotype IgG responses persisted for at

Figure 1. Side effects observed during idiotype vaccination. A, percentage and severity of side effects per total of 65 idiotype vaccinations. B, percentage and severity of side effects per total of 18 vaccinated patients. * thrombocytopenia and anemia grade 3 were observed in one patient each. Both patients had formal grade 2 cytopenia before vaccination.

Figure 2. Immune status and detection of vaccine-specific cellular immune responses by ELISPOT. A, T-cell subsets in the peripheral blood of vaccinated patients according to diagnostic subgroups and fludarabine pretreatment. Hatched areas represent normal ranges. F, fludarabine. *, a case with leukemic mantle cell lymphoma (A32). B, columns, mean of IFN\textgamma spots obtained from individual patients against the vaccine (patient Fab) and a randomly chosen control Fab; bars, SD. Patient A60 has a strictly specific immune response, whereas patient A15 displays partial cross-reactivity. C, T-helper cell counts of patients according to detection or absence of a cellular immune response as determined by ELISPOT. *, leukemic non-Hodgkin’s lymphoma (four CLL and one mantle cell lymphoma). Pos., positive; neg., negative. D, combined statistical analysis of all patients compared with prevaccination reactivity to control Fab (with 95% confidence interval) by a Poisson regression model. Pre, before vaccination; Post, after vaccination.
least 48 weeks in two patients (A21 and A26) without disease progression during this period (Fig. 5C).

**Clinical outcome.** Six patients were taken off study before the fourth immunization due to early disease progression. Six patients received one to four additional compassionate use immunizations in monthly intervals (Table 1). Six patients have died from lymphoma 4 to 38 months after start of vaccination therapy.

Patients with an anti-idiotype immune response tended to have a better progression-free survival than immunologic nonresponders (Table 1), although the heterogeneity of the patient population in this phase I trial design does not permit meaningful statistical correlations. A quantitative analysis of disease variables in evaluable patients and seemed to occur independently of the patient’s cellular immune status. This experience is encouraging efficacy, experimental evidence suggests that immunization with unglycosylated antigen may not impair but rather enhance cellular immune responses against naturally glycosylated peptides (30, 31).

With respect to vaccine administration, intradermal vaccination at a GM-CSF-conditioned site was chosen to deliver the antigen to professional APCs of the skin. GM-CSF is a potent adjuvant for induction of antigen-specific T cells through stimulation of bone marrow–derived APCs (6, 32). This immunization route was adopted explicitly to induce cellular immunity because cellular immune responses induced by idiootype vaccination with GM-CSF was associated with clearance of MRD (10), and because our own in vitro data showed efficient induction of MHC-restricted, idiotype-specific CDS+ CTL by idiotype-presenting dendritic cells, the most potent APC type known (21).

Finally, our vaccine was not conjugated to KLH as in most trials (9, 10, 18) because conjugation rates were highly variable and failure to fulfill GMP criteria. Instead, the vaccine was formulated with the potent lipid-based adjuvant MF59 (23), which has not yet been evaluated for antitumor vaccination. Because this trial tested, to our best knowledge for the first time, the parenteral application of proteins expressed individually in *E. coli*, a phase I design with feasibility and safety as primary end points was adopted. Although the vaccination was generally well tolerated, the main disadvantage of the trial design was the inclusion of heavily pretreated patients with a strongly impaired immune system as indicated by low numbers of circulating T-helper cells and refractoriness to hepatitis B vaccination. In contrast to our study population, reported response rates to two hepatitis B vaccinations, defined as anti-HBs titers of $\geq 10$ IU/mL, vary between 86% in a healthy control group (mean age, 57 years), and 41% in breast carcinoma patients in remission at 8 weeks (33).

Despite this profound immunosuppression, humoral and/or cellular immune responses against idiotype developed in 10 of 17 evaluable patients and seemed to occur independently of the patient’s cellular immune status. This experience is encouraging.

**Discussion**

This first clinical application of a novel idiotype vaccine differs in several aspects from conventional idiotype vaccination approaches: technologically, the production strategy is based on A-PCR, and minute amounts of tissue suffice as starting material. The expression strategy as a recombinant Fab fragment in *E. coli* (20) has a success rate of $\sim 90\%$ and permits substantially reduced manufacturing times. Although lack of glycosylation of these bacterially expressed vaccines could potentially affect their
because the humoral immune response to a KLH-conjugated s.c. vaccine seems to be dependent on B-cell recovery after passive immunotherapy with anti-CD20 antibody (34). The strong immunogenicity of our vaccine formulation is further emphasized by the fact that seven of the immune responders failed hepatitis B vaccination.

The development of trace amounts of autoantibodies in some patients cautions against the use of even stronger adjuvants to avoid the induction of autoimmune disease. On the other hand, no disease exacerbation was noted in a patient with concomitant rheumatoid arthritis.

A predominant specificity of the cellular immune response towards the individual idiotype was shown by stringent analysis of ELISPOT results with a statistical regression model. This model assesses induction of increased specific reactivity by appropriately accounting for actual reactivity against control targets at every time point. Thus, this calculation performs an objective comparison between pre- and post-vaccination samples without requiring prior definition of thresholds to correct for numbers of spots in negative (no target) and positive (lectin induced) control wells. A lesser increase of nonspecific T-cell reactivity observed against random control targets may be explained by reverse immunology through shared epitopes in vaccine and control target and seems to be dependent on their degree of similarity. Therefore, the specificity of an anti-idiotype immune response may be "relative" and to depend critically on the control target analyzed.

Further probing into the nature of idiotype specificity by epitope mapping revealed that the only peptide that was recognized statistically better than the control was derived from a CDR region. This assay with peptide targets also confirms the idiotype reactivity with a different antigenic format. The inferior response observed against C region peptides is consistent with the assumption that individual rather than shared idiotype epitopes are better targets for cellular immune responses because strong tolerance can be expected for these ubiquitous C region epitopes.

These results are noteworthy because T cells with specificity for FR-derived peptides are present in the blood of lymphoma patients and can be activated in vitro (35). In contrast, little information is available on the precise specificity of in vivo–induced immune responses to idiotype vaccines. Our data indicate that CDR-derived rather than FR epitopes can represent preferential targets of MHC class I–restricted T cells after activation in vivo. In addition, MHC class II–restricted CDR peptides have been identified as cellular immune targets upon vaccination with KLH-conjugated idiotype immunoglobulin and GM-CSF (36), thereby strengthening the conclusion that individual rather than shared idiotype features are preferential targets for vaccination-induced immune responses. Whether cellular immunity indeed represents the most relevant anti-idiotype effector mechanism is not yet clear because a correlation between FcγR III genotype and outcome of follicular lymphoma patients after idiotype vaccination suggests an important role for humoral anti-idiotype immunity (12). Nevertheless, it may be assumed that a T-helper cell response may be required for efficient induction of anti-idiotype antibody responses.

Because the A-PCR-based production strategy of the recombinant idiotype Fab fragment requires only small lymphoma biopsies, we generally obtained insufficient material for in vitro testing for cellular or humoral immune reactivity against lymphoma cells. Killing of autologous lymphoma cells by idiotype vaccination-induced immune cells has, however, already been described (10). Because our own in vitro experiments have also established MHC class I–restricted, specific killing of idiotype-expressing target cells by T cells stimulated with recombinant Fab idiotype (21), it may be assumed that the cellular immunity induced by our vaccine should also be able to attack actual lymphoma cells.

In conclusion, the results of this trial indicate feasibility, good tolerability, and potent immunologic activity of the recombinant idiotype vaccination strategy tested. Immune responses to the vaccine were induced irrespective of the cellular immune status or lack of response to a conventional anti-infection vaccine, indicating that our vaccine formulation may overcome even profound clinical immunodeficiency. These results warrant further clinical testing in earlier disease stages and more homogeneous patient cohorts to assess clinical efficacy. Possible induction of autoimmunity has been identified as a potential hazard of strong vaccine immunogenicity and calls for monitoring of autoimmunity in clinical trials testing potent novel cancer vaccines.

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