

Anti-idiotypic vaccination in the treatment of low-grade B-cell lymphoma

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Background and Objectives. Patients with B-cell lymphoma can be induced to mount a specific immune response against the individual idiotypic determinants expressed in their tumor cells. This form of active immunotherapy is now under evaluation in the clinical setting. We evaluated the feasibility and effectiveness of this kind of immunotherapy in a group of patients with low-grade lymphoma, which included two cases of bi/triclinal lymphoma.

Design and Methods. Nine patients with a histopathologic diagnosis of follicular non-Hodgkin's (NHL) low-grade B-cell lymphoma were initially selected for this disease-free survival study. Idiotypic proteins were recovered by somatic fusion of the tumor cells and their identity with the tumor idiotype determined by molecular methods. The patients received the vaccine consisting of their tumor Ig protein coupled to keyhole limpet hemocyanine and were observed for toxicity, anti-idiotypic immune response, clinical outcome and circulating t(14;18)⁺ tumor cells.

Results. The median duration of follow-up was 40 (10-64) months from the initiation of immunotherapy. Tumor regression was detected in two patients. No tumor progression was observed in the other patients. Eight patients generated specific anti-idiotypic antibodies and 3 out of five were cleared of circulating t(14;18)⁺ cells.

Interpretation and Conclusions. Induction of tumor-specific anti-idiotypic immune responses may be of benefit to patients affected by low-grade B-cell NHL. Our results are in line with those previously reported and call attention to the issue of tumor clonality in this kind of treatment.

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Key words: NHL immunotherapy, idiotypes, bclonal lymphomas, B-cell lymphomas, tumor vaccines

The majority of patients with low-grade follicular lymphomas relapse and cannot be cured by standard chemotherapy and radiotherapy. A different therapeutic approach to this kind of B-cell malignancy involves the induction of immune responses by vaccination with tumor-specific antigens. The immunoglobulins synthesized by B-cells and related tumors contain idiotypic determinants that have been located in the variable regions of both immunoglobulin peptide chains, a region of this molecule that normally provides the recognition sites for foreign antigens but that can itself be recognized as an antigen by other immune cells.¹ Given the singularity of the idiotypic region of each B-cell, induction of the immune attack targeted at idiotypic determinants can lead to destruction of the tumor cells while sparing normal B-cells.

Although promising results were initially obtained with the infusion of murine anti-idiotypic monoclonal antibodies in low-grade lymphoma patients,² a substantial proportion of these patients relapsed with idiotope-variants of the original tumor.³ In the light of these problems, an active immunization alternative, aimed at stimulating a polyclonal response^{4,5} that would reduce the incidence of escaped mutants, was evaluated after vaccination of patients with an immunogenic preparation of their own tumor idiotype (Id).⁶ The seminal paper by Ronald Levy's group was later followed by a wider study by his, and related groups, all based in U.S. centers.⁷⁻⁹

Here we present the results of a clinical trial of active immunization in patients with low-grade follicular lymphoma, this being, to the best of our knowledge, the first conducted in Europe. We report the results obtained in nine patients with a median follow-up at this time of 40 months. The vaccination protocol consisted of a series of injections of the autologous idiotypic protein conjugated to a highly immunogenic foreign carrier pro-

tein. In this report we discuss the methodology, the clinical outcome and variations of the protocol related in particular to the impact of clonality on the implementation of the treatment.

Design and Methods

Patients

All patients had a histopathologic diagnosis of follicular non-Hodgkin's low-grade B-cell lymphoma type I or II according to the REAL classification¹⁰ and were in clinical stage IV. Twelve patients were initially selected for this disease-free survival study, nine of whom have completed the treatment at the time of this report. Patients with a serum monoclonal band or with a HIV-positive test were not eligible. The trial was approved by the Hospital's Ethical Board and communicated to the Spanish Ministry of Health authorities. The study was carried out in accordance with the Declaration of Helsinki (1983 version). The patients were informed about the background and current knowledge concerning the proposed approach before obtaining their oral and written consent. A lymph node biopsy was taken from each patient for the purpose of producing the idiotypic vaccine. Patient #6 presented with leukemic dissemination and blood was used as the source of tumor cells.

Table 1 shows the characteristics of the nine patients who completed the treatment. Patients #1-6 had been treated with several chemotherapy lines because of repeated disease relapses and had undergone autologous hemopoietic transplantation. After the treatment these 6 patients were examined for the presence of complete remission (CR) or partial remission (PR) of the disease and were vaccinated with idiotypic vaccine. Patient #7 received the idiotypic vaccine after achieving first complete remission with front line chemotherapy. Patients #8 and 9 received several chemotherapy lines (SCL) and were vaccinated whilst being in partial remission. None of the patients had received any chemotherapy for at least six months before vaccination or anti-tumor therapy during the study, except patient #3 (see *Results*).

All patients were staged on the basis of CT scans of the chest, abdomen and pelvis and with bone marrow trephine biopsy before vaccine treatment. Blood counts, blood chemistry, chest radiographs and CT scans of the chest, abdomen and pelvis were performed 3 times a year and the patients were surveyed for the presence of autoantibodies and tumor recurrence according to a standardized protocol. The patients were observed for toxicity, humoral anti-idiotypic responses, clinical outcome

Table 1. General characteristics of the patients.

Patient	Age*/Sex	Diagnosis	Stage	Tumor isotype	Previous therapy
1	37/F	FL type II	IV	IgG λ	SCL + ABMT
2	43/M	FL type II	IV	IgG λ	SCL + ASCT
3	43/M	FL type II	IV	IgG κ , IgM κ	SCL + ASCT
4	30/F	FL type II	IV	IgG λ	SCL + ASCT
5	29/M	FL type I	IV	Biclonal IgM κ	SCL + ASCT
6	36/F	FL type I	IV	IgM κ	SCL + ASCT
7	62/M	FL type II	IV	IgG λ	CVP
8	31/M	FL type II	IV	Tricl. IgM κ/λ , IgG λ	SCL
9	31/M	FL type I	IV	IgG λ	SCL

*At diagnosis. SCL: several chemotherapy lines. CVP (cyclophosphamide, vincristine and prednisone); ABMT (autologous bone marrow transplant) ASCT (autologous peripheral blood stem cell transplant).

and presence of circulating t(14;18) positive lymphoma cells detectable by a polymerase chain reaction (PCR) assay.

Cell lines and reagents

The HAT-sensitive heteromyeloma K6H6/B5 (CRL-1823), the anti-human μ -chain hybridoma DA4-4 (HB-57) and the p(14;18)⁺ non-Hodgkin's lymphoma cell line CL (CRL-2261) were obtained from the American Type Culture Collection. Fluorochrome-labeled anti-human μ , γ , κ , λ and anti-CD19 antibodies were from Caltag Laboratories (Burlingame, CA, USA).

Cell preparation

Cells from involved lymph nodes (LN) were obtained at the time of diagnosis and single cell suspensions were prepared for cytofluorometric analysis and somatic fusion procedures. Cells were stained by standard direct immunofluorescence techniques and analyzed using an EPICS XL (Coulter Electronics, Hialeah, FL, USA). Peripheral blood mononuclear cells (PBMC) were isolated by Lymphoprep density-gradient centrifugation (Nycomed Pharma AS, Oslo, Norway).

LN cells or PBMC (in patient #6) were fused in 50% polyethylene glycol (Boehringer Mannheim, Germany) with K6H6/B5 cells. The resulting hybrids were screened for production of human Ig by a conventional class-specific enzyme-linked immunosorbent assay (ELISA).¹¹ An initial selection of hybrids producing the same Ig (H+L) was performed after studying the electrophoretic mobility of the secreted idiotypes by agarose electrophoresis, capillary transfer to PVDF membranes and staining with HRP-labeled anti-Ig reagents (Sigma-Aldrich, St. Louis, MO, USA) and chemiluminescence (Boehringer Mannheim, Mannheim, Germany).

Identification of the idiotypic tumoral Ig

Several DNA-based strategies were used in order to confirm the identity of the hybridoma products and the Id present in the tumor samples. cDNA was synthesized from total RNA samples (S.N.A.P. Kit, InVitrogen, Carlsbad, CA, USA) using an oligo(dT)-primer and AMV reverse transcriptase (Promega, Madison, WI, USA) and amplified using a panel of V_Hback/J_Hfor family-specific oligonucleotide primers.¹² For PCR-RFLP restriction analysis, the purified PCR products were digested with *Hae*III and run on a 8% polyacrylamide gel. In some special cases when the information gained after PCR-RFLP analysis was not conclusive, the PCR products were subcloned into pCRTMII using the TA cloning system (InVitrogen) and introduced into chemically-competent bacteria. The sequence of the inserts was determined by using the Cy5TM AutoCycleTM Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) on a Pharmacia Biotech ALFexpressTM Automater DNA Sequencer.

Bcl-2/IgH gene translocation

PCR amplifications of the t(14;18) breakpoint regions were performed using nested oligonucleotides.¹³ Genomic DNA (1 µg) was amplified for 30 cycles in PCR buffer (50 mmol/L KCl, 10 mmol/L TRIS, pH 8.3) containing 200 µmol/L dNTPs (Pharmacia Biotech), 2.5 mmol/L MgCl₂ and 1.25 U of AmpliTaq Gold polymerase (Roche Molecular Systems, Branchburg, NJ, USA) in a final volume of 50 µL with the following cycling: 94°C, 45s; 58°C, 45s; 72°C, 1 min, and a 10 min final extension at 72°C. A 5 µL aliquot was then re-amplified for 30 cycles in a 50 µL volume using internal primers. Aliquots of the final product were separated by electrophoresis in 2% agarose/ethidium bromide. For each sample, at least two separate amplifications were performed. The quality of the template DNA was assessed by amplification of a 600-bp fragment of the human GAPDH gene (Stratagene, La Jolla, CA, USA). The bcl-2 assay detected one rearranged genome in 10⁵ cells of the t(14;18)⁺ CL cell line.

Vaccine preparation

Hybridoma cells secreting the tumor idiotypic protein were grown at high cell density in RPMI 1640 (Bio-Whittaker Europe, Verviers, Belgium) supplemented with 10% fetal calf serum (FCS) (PAA Laboratories, Somerset, UK), L-glutamine and antibiotics (GibcoBRL, Paisley, Scotland) by using CL1000 chambers (INTEGRA Biosciences, Wallisellen, Switzerland). The purification of the tumor Id protein was accomplished by means of affinity

chromatography techniques that exploit the individual absorptive properties of each immunoglobulin: ProteinA-Sepharose (Pharmacia Biotech) (IgG), ProteinL-Sepharose (Actigen, Cambridge, UK) (κ chain) or DA4-4(anti-μ)-AminoLink Plus (Pierce, Rockford, IL, USA) (IgM). General safety rules were observed in all the processes and the purity of the final products was determined by SDS-PAGE and agarose electrophoresis.

Equal volumes of purified idiotypes and keyhole limpet hemocyanine (KLH, Endotoxin-free, Calbiochem, San Diego, CA, USA) both at 1 mg/mL of PBS were coupled in 0.1% glutaraldehyde and dialysed against saline solution in sterile Slide-A-Lyzer cassettes (Pierce). The final product was checked for endotoxin levels and microbial contamination and stored at -80°C until use. The adjuvant SAF was prepared by homogenizing a mixture of Pluronic L-121 (BASF Corporation, Parsippany, NJ, USA), Squalane and Tween 20 (both from Sigma) in PBS in a Polytron machine.¹⁴ The patients received a series of five monthly subcutaneous vaccinations with Id (0.5 mg)-KLH conjugates emulsified in an equivalent volume of SAF adjuvant (patients #1 to #6) or mixed with 300 µg of human granulocyte-macrophage colony-stimulating factor (GM-CSF) (Novartis Pharma, Basel, Switzerland) (patients #7 to #9). An identical booster vaccination was given three months later. The patients treated with GM-CSF received, for each course of vaccination, three additional cytokine doses administered daily around the vaccination point.

The immunogen for patient #5 was prepared with a mixture of the two idiotypes present in the tumor sample coupled to KLH. In patient #9, who had a triclinal lymphoma, the IgG and a mixture of the IgMκ and IgMλ idiotypes were separately coupled to KLH.

Humoral response evaluation

Anti-idiotypic and anti-KLH circulating antibodies were determined in an ELISA. Pre- and post-immunization sera were serially diluted and dispensed in wells of microtiter plates coated with purified idiotypes or KLH. Bound antibodies were detected with affinity-purified horseradish peroxidase (HRP)-conjugated goat anti-human anti-serum (Sigma) against the light chain isotype not present in the corresponding idio-type. Absorption was evaluated at an optical density of 490 nm. A three-fold increment of the optical density readings over the negative control (unrelated idio-type) was considered to be a positive response.

Results

Vaccine production

To establish the tumoral origin of the hybridoma idiotypes as early as possible, we first chose those hybrids which secreted immunoglobulins with the expected light and heavy chains and shared the same electrophoretic mobility. The VH regions were then expanded by RT-PCR and the electrophoretic pattern of *HaeIII*-digested material compared with that obtained from tumor samples. Nucleotide sequencing of the expressed VH genes was conducted to confirm idiotypic identity in those cases in which RFLP analysis was inconclusive due to technical problems or biconality. A vaccine was successfully manufactured in all the attempted cases.

Tumor clonality

In theory, correct identification of a tumor VH sequence depends on the repetition of a single predominant sequence which is presumed to derive from the lymphoma cells. The VH sequence coding for the lymphoma idotype must be recognizable in the hybridoma cells. Table 2 shows the expressed isotype and the VDJ used by the tumor cells and hybridomas derived from certain special cases. Case #7 showed one predominant sequence in both the hybrids and the tumor samples. Sequencing did not reveal intraclonal variation. In case #3, the previous cytofluorometric analysis of lymph node cells revealed the presence of 70% of CD19⁺ cells expressing IgG κ and IgM κ . However, only one VH sequence was found in either the tumor sample or in the IgG or IgM-producing hybridomas, indicating the presence of a single idotype and a monoclonal tumor with two switch-related variants. In case #5, although only one population of IgM κ was present in the tumor sample, two V_H consensus sequences were found. These sequences were individually expressed by the hybrids, which indicates the presence of two clonal populations in the tumor. Light chain sequencing of the hybrids and tumor samples confirmed the suspicion of biconality.¹⁵ In patient #8, suspensions of tumoral lymph nodes contained two subsets of CD19⁺ cells staining with IgM (25%) and IgG (13%). The analysis of 155 hybridoma supernatants revealed that 20% secreted IgM κ , 40% IgM λ and 40% IgG λ . Amplification of the tumor cDNA with V_H-C μ or C γ -specific primers and PCR-RFLP analysis of *HaeIII* digests demonstrated the presence of two different patterns. Detailed sequence analysis showed that the IgM κ and IgM λ -secreting clones used the same V_H and J_H gene segments but differed in their

Table 2. Phenotype of tumor cells and VH usage from patients #3, 5, 7 and 8.

Patient n.	Tumor Ig	VH	D-J
3	IgG κ , IgM κ	V1-69	D5-5/JH4b
5*	IgM κ	V4-61 V4-39	D2-21/JH6b DN1/JH4b
7	IgG λ	V3-30	D3-3/JH3b
8°	IgG λ IgM κ IgM λ	V3-15 V1-02 V1-02	D2-15/JH6b D1-7/JH4b D1-26/JH4b

The sequences of several clones were compared using the PCGENE program v6.85 and the search for homologous germline sequences was done in the V BASE Directory of Human V Gene Sequences (MRC for Protein Engineering Center, Cambridge, UK). *Biclonal IgM κ tumor; °triclinal IgG λ , IgM κ , IgM λ tumor.

use of the D fragment, whereas the IgG λ -secreting hybridomas used a different set of V-D-J germline genes (Table 2). PCR-RFLP analysis of hybridoma-derived cDNA was consistent with the corresponding, isotype-matched pattern found in tumoral samples. Altogether, these data indicate the presence of a triclinal tumor in patient #8.

Antibody responses

Serum collected prior to and at different times during the course of the immunization was analyzed for the presence of anti-idiotypic or anti-KLH antibodies. Post-vaccination responses specific to the autologous idiotypes were detected in 8 of the 9 patients (Table 3). Patient #7 exhibited only a mild anti-idiotypic response that did not meet the criteria for positivity. In patients with biconal and triclinal lymphomas, the antibody responses were detected against all the individual idiotypes. Positivity was first detected after three injections of the vaccine and high levels of anti-idiotypic antibodies persisted for at least six months after the last injection. All of the patients developed anti-KLH antibodies (*data not shown*).

Toxicity and clinical outcome

Nine patients completed the planned immunotherapy course. The toxicity of the treatment varied and consisted mainly in mild local reactions at the site of injection (erythema and induration). Systemic symptoms were rarely observed and they consisted of transient low-grade fever and general discomfort. No hepatic, renal, pulmonary, cardiac, hematologic, gastrointestinal, or neurologic toxicity was observed. Antinuclear antibody tests were negative. Transient elevations of rheumatoid

Table 3. Clinical outcome, anti-idiotypic response and presence of circulating t(14;18) positive cells in vaccinated patients.

Pat.	Clinical situation		Follow-up* months	Anti-Id response		t(14;18) cells	
	Pre-vaccine	At present		Prevaccine	Postvaccine	Pre-vaccine	Postvaccine
1J	PR	Exitus	14 months	+		ND	
2J	CR	CR	64 months	+		+	-
3J	PR	CR	56 months	+		+	-
4J	CR	CR	50 months	+		-	
5J	CR	CR	37 months	+**		-	
6J	CR	CR	19 months	+		+	-
7 [#]	CR	CR	43 months	+/-		+	+
8 [#]	PR	PR	13 months	+**		+	+
9 [#]	PR	PR	10 months	+		-	

PR: partial remission. CR: complete remission. *From the initiation of vaccination; **anti-idiotypic response to all the immunizing idiotypes; J: vaccinated with SAF adjuvant; [#]vaccinated with GM-CSF.

factor titers were occasionally found, but no signs of related diseases were observed.

Five patients were in complete clinical remission and four had residual disease before vaccination. The median follow-up of the patients is 40 months (range 10-64) from the initiation of vaccination (Table 3). Patient #1 developed a specific immune response and underwent complete tumor regression, but died from an unrelated disease 14 months after initiation of vaccination. Patient #3 showed transient tumour regression 3 months after initiating vaccination but relapsed 7 months later. The relapse was treated with oral cyclophosphamide for 10 months. Evidence of tumor regression was observed three months after initiation of chemotherapy and the patient is currently in complete clinical remission 54 months after vaccination.

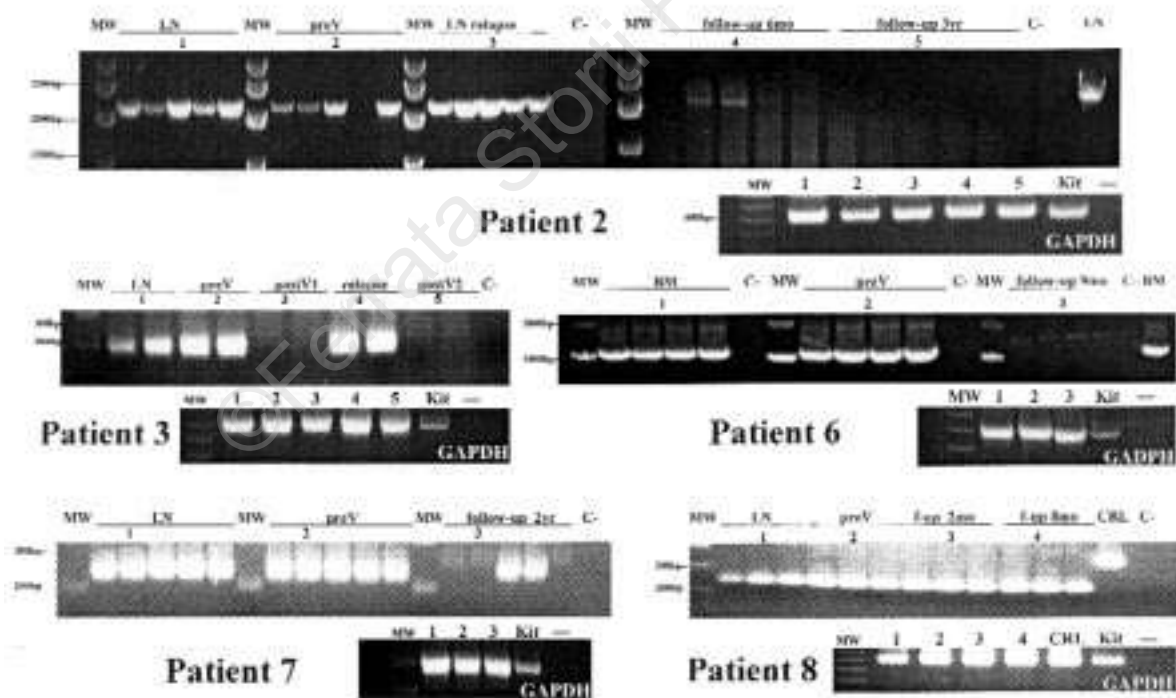


Figure 1. Monitoring of bcl-2/IgH translocation in samples of lymph nodes (LN), bone-marrow (BM) or peripheral blood mononuclear cells that were collected immediately before (preV) or after vaccination (postV, follow-up). Patients #2 and #6 presented a mcr rearrangement. Patients #3, #7 and #8 presented MBR rearrangements. For patients #2, #6, #7 and #8, the times elapsed after the last immunization are indicated. The second lymph node sample from patient #2 was obtained at the time of relapse, between the fourth and the fifth vaccination doses. In patient #3, PBMC were collected at the time of the fifth Id-KLH injection (postV 1), and three (relapse) or four months (postV 2) later. Data represent PCR products of a different number of replicates. The GAPDH gene was amplified as an internal control. MW: molecular size markers. C-: DNA from a normal subject. Kit: control amplification. CRL: DNA from a bcl-2/IgH positive cell line.

Patient #2 relapsed between the 4th and 5th vaccine dose. The patient completed the vaccination course. Although tumoral masses revealed by CT persisted for a period of six months, they diminished and disappeared without any further treatment and there is no evidence of tumor relapse at present. Four of the five patients in remission before vaccination exhibited anti-idiotypic immune responses and remain in remission at the time of this report.

Minimal residual disease

Rearranged bcl-2 positive tumor cells detectable for PCR were studied in tumor samples and PBMC (Figure 1). Despite ASCT and/or conventional chemotherapy, cells from the malignant clone were detected by PCR in the blood of five patients before vaccination. In patient #7, translocation-bearing cells may have decreased in number but did not disappear, since only three out of five expansions were positive after vaccination. Despite the presence of tumor cells in the circulation, this patient remains in clinical remission. In patient #2, a decrease in the number of translocation-bearing cells was evident after completion of the initial doses, despite the recurrence of lymphadenopathy that appeared after the fourth dose. The patient's blood remained negative for the lymphoma marker at least three years after vaccination. In patient #3, circulating t(14;18)⁺ cells were not detected by the time of the fifth dose, but reappeared during the relapse of the tumor three months later. Following chemotherapy, the PCR assay excluded the presence of t(14;18)⁺ cells. In patient #6, the positive amplification reactions present in bone-marrow and PBMC samples at diagnosis disappeared after treatment (follow-up, 9 months), whereas in patient #8, t(14;18)⁺ cells were still detectable in blood samples taken 10 months after vaccination.

Discussion

The optimal treatment for low-grade NHL remains to be determined. Overcoming the ineffectiveness and potentially harmful effects of classical chemotherapy and radiotherapy treatments has been the aim of alternative tumor therapies, such as immunotherapy. Certain immunologic and clinical features of low-grade B-cell lymphomas make them especially attractive as targets for the application of this innovative approach. Some such features are a well-defined tumor-associated antigen (the idotype) expressed in the context of a professional antigen-presenting cell and the relatively indolent course of the disease over a long period of time.

A vaccine based on the unique idiotypic protein expressed by each patient's tumor was produced by means of hybridoma technology and manufactured at the time of recovery from cytoreductive therapy. Early identification of the tumor Id among the hybridoma idiotypes and the use of high-density cultures and affinity techniques for protein isolation permit the completion of the process in a six-month period.

The issue of clonality must be borne in mind throughout the whole process. It is worth noting that in our study 2 out of 12 patients showed bi/tri-clonal tumors. Their individual idiotypes had to be isolated from selected hybridomas and the vaccine contained different combinations of the idiotypic proteins coupled to KLH. Although those figures are not far from the 10% reported frequency of biclonal lymphomas,¹⁶ it is remarkable that none of the NHL patients already immunized in other centers was reported to have biclonal tumors. In any case, our findings highlight the importance of defining the clonality of tumor samples in the context of idiotypic vaccination.

Two of the patients who were in PR (patients #1 and #3) or relapsed during treatment (patient #2) experienced complete remission after vaccination. Patients #4 to #7 remain in their initial CR condition, even patient #7 who showed a comparatively minor anti-idiotypic response. No tumor progression occurred in the two other patients in PR (patients #8 and #9). Investigation of the presence of disease at molecular level showed that 3 out of the 5 t(14;18)-positive patients were cleared of translocation-bearing cells. Two of them were receiving the idiotypic vaccination as the only treatment for their disease, whereas patient #3 received additional oral chemotherapy. The achievement of molecular remission in this patient is remarkable, given that oral chemotherapy alone does not usually result in elimination of translocation-bearing cells in the periphery. It is possible that both treatments contributed to the clinical outcome in patient #3.

All the patients appeared to be immunocompetent, as shown by their ability to mount immune responses to the carrier molecule KLH and in most cases to their own idiotypic proteins. Although the induction of anti-idiotypic T-cells was not investigated in this study, it has been previously reported that most immunized patients develop humoral and cellular anti-idiotypic responses simultaneously.^{6,9} It is still too early to evaluate the impact that the use of SAF adjuvant or GM-CSF may have had on the outcome of anti-idiotypic vaccination.

We have not found differences in the titers of anti-idiotypic antibodies induced in patients treated with either SAF or GM-CSF. GM-CSF is a pleiotropic cytokine that promotes the differentiation of progenitor cells to functional dendritic cells. It may be the adjuvant of choice in further trials, because of its availability and safe use in humans.¹⁷

The median duration of follow-up was 40 months from the initiation of vaccination. At present it is difficult to predict whether the patients will enjoy a longer relapse-free period and higher overall survival compared with those receiving conventional treatments. Our results confirm previous reports showing the induction of specific anti-idiotypic responses and extend these findings to more complex cases of bi- or triclinal lymphomas. The evidence of reduced tumor burden, the presence of anti-idiotypic immunity and the clearance of t(14;18)⁺ cells in some patients suggests that the treatment could be clinically relevant. Clearance of t(14;18)⁺ cells from peripheral blood cells may precede the disappearance of residual tumor cells in other lymphatic tissues or may be at least an indication of their dormancy. The possibility of a definitive cure in those patients whose translocation-bearing cells disappear after the vaccination is a likely prospect.⁹ It is possible that the treatment described here may be more useful for patients who are in their first clinical remission. It should be emphasized that some of these patients were previously undergoing more aggressive therapeutic treatments and suffered a relapse of their disease.

To expand the scope of individualized NHL therapies, the source of vaccine must be generated in a time-frame of weeks rather than months or years. Alternatives based on the use of single chain Fv vaccines¹⁸ effectively blocked tumor progression in mouse models of lymphoma,^{19,20} although their superiority over conventional immunotherapy with the conjugated idiotypic has not been proven. New vaccine formulations including the use of idiotypic-pulsed dendritic cells^{21,22} will eventually contribute to the development of stronger immune responses.

Another alternative that recalls earlier attempts of passive immunization, and has emerged since the humanization of monoclonal antibodies directed against B-cell specific molecules, can be applied in a greater number of patients.²³ Anti-idiotypic vaccination has the advantage of sparing the normal B-cells and its effect must necessarily be longer lasting. In any case, additional studies in larger series of patients are necessary to prove the existence of a relationship between anti-idiotypic immunity and improved clinical outcome.

Contributions and Acknowledgments

YB performed the initial laboratory experiments pursuing the vaccine preparation and molecular analysis, participated in the revision of the final version, and should be considered as principal author. RY took over those responsibilities in the last patients studied and performed the long follow-up studies. AP participated in the supervision of the molecular studies. RC and FDE were responsible for the conception of the study, direct supervision, data collection and manuscript preparation. M-NF, RF and MB performed the studies at diagnosis and contributed to the clinical management of the patients. Authors are listed according to their decreasing contribution to this work, while the last two authors shared a major role as senior authors.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlap with previous papers.

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PEER REVIEW OUTCOMES

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Gilles Salles, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Salles and the Editors. Manuscript received December 6, 2001; accepted February 14, 2002.

What is already known on this topic

Anti-idiotypic vaccination has been reported to be effective in follicular lymphoma patients. A limited number of patients have been treated.

What this study adds

This study reports another trial in 9 patients using KLH-coupled Ig protein and adjuvant. Two of them had objective tumor response and prolonged clinical and molecular remissions were observed in several patients.

Potential implications for clinical practice

Vaccination in follicular lymphoma using idio-type-based protein is feasible and appears to be effective.

Gilles Salles, Associate Editor