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Active idiotypic vaccination in a patient with biclonal follicular lymphoma

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Abstract Specific immunological responses to the idiotypic epitopes present in the surface immunoglobulin (Ig) of the clonal tumour population can be induced for active immunotherapy in patients with B-cell non-Hodgkin lymphoma (NHL). The clonality of the tumour cells should have important implications for the success of the implemented therapy. Here we report on the case of a patient enrolled in a protocol of active idiotypic immunotherapy in which previous cytofluorometric analysis showed a major IgM^+, κp^+ population in the tumoral cell suspensions. However, sequence analysis of both tumour sample and tumour-derived hybrids revealed the presence of two unrelated clones that used different VH and VK gene segments. It was possible to obtain hybridomas secreting these two different IgM, κp idiotypic proteins. The patient was immunised with a mixture of these two idiotypic Igs conjugated to keyhole limpet haemocyanin. Anti-idiotypic antibodies directed against both tumour-associated proteins were detected. This is the first case of anti-idiotypic therapy in a patient with a biclonal NHL. Our work calls attention to the question of clonality in the context of idiotypic vaccination in NHL patients.

Key words Biclonal lymphoma · Idiotypic immunotherapy · Tumour vaccines

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Introduction

Neoplastic disorders are generally believed to arise from a single malignant clone with genetic alterations that permit uncontrolled proliferation [9]. In the case of B-cell lymphomas, the use of methods to detect immunoglobulin (Ig) variable region variations in selected subpopulations of tumoral cells revealed that the incidence of biclonal tumours may be as high as 10% [15]. On the other hand, it is important to differentiate a truly biclonal tumour from tumour heterogeneity arising from the frequent finding of clonal evolution [1, 4]. The presence of two different populations of tumour cells has important implications in the application of a new kind of treatment that relies on the active immunotherapy of B-cell lymphoma patients by vaccination with the idiotypic protein expressed on the surface of the neoplastic cells [5, 12]. Current methodology implies the rescue of the idiotypic protein by somatic fusion of tumour cells and an appropriate myeloma and the subsequent determination of the identity between the hybridoma products and the patient's tumoral immunoglobulin.

Here we report the case of a patient with an IgM, κp, B-cell lymphoma in which the sequence analysis of the variable region of both heavy- and light-chain gene segments used by the tumour and tumour-derived hybridoma cells showed a dual VH4 (V4–61)/DPK9 and VH4 (V4–39)/DPK1 germ-line origin, indicating the presence of a biclonal tumour. This finding does not by itself preclude active anti-idiotypic therapy because, as we show in this case, it is possible to rescue the idiotypic immunoglobulin present in both malignant clones and induce a specific humoral response to both tumour products.

Material and methods

A 43-year old man with a low grade B-cell lymphoma (follicular small cleaved cell type, according to Working Formulation), clinical stage IV, was treated with several chemotherapy lines (CVP,

CHOP, IMVP-16) and local radiotherapy because of different relapses of lymphoma. An autologous stem cell transplantation was performed (conditioning treatment TBI plus cyclophosphamide) and resulted in complete remission for a 2-year period. A new relapse led us to include the patient in a protocol of active immunotherapy with the autologous tumour idiotypic protein developed at our centre (manuscript in preparation). The trial included 12 patients, and was approved by the Hospital's ethical committee and communicated to the Spanish Ministerio de Sanidad authorities. The study was carried out in accordance with the Declaration of Helsinki (1983 version). The patients' oral and written consents were obtained after they had been informed about the background and present knowledge of the proposed approach.

A new complete remission was achieved after treatment with chlorambucil and prednisone 9 months before vaccination. Single-cell suspensions from a lymph node (LN) biopsy were prepared for cytofluorometric analysis and fusion procedures. Cells were stained by standard direct immunofluorescence techniques with fluorescent-labelled anti-CD19, anti-μ chain, anti-κ chain, anti-λ chain and anti-γ chain antibodies (Caltag Laboratories, Calif., USA) and analysed by an EPICS XL (Coulter Electronics, Hialeah, USA). LN cells were fused in 50% polyethylene glycol (PEG, Boehringer Mannheim Biochemical, Mannheim, Germany) with the HAT-sensitive heterohybridoma K6H6/B5 (American Type Culture Collection, CRL 1823). The resulting hybridomas were initially screened by an enzyme-linked immunosorbent assay for the production of Ig matching the isotype of the tumour [3] and cloned by limiting dilution.

Sequence analysis of the tumour and hybridoma VH and VL genes

Total RNA was extracted from 1×10^6 LN cells or 0.5×10^6 hybridoma cells (S.N.A.P. total RNA isolation Kit, InVitrogen, Calif., USA). cDNA synthesis was performed with AMV reverse transcriptase and oligo(dT) primer (Promega, Wis., USA). This cDNA was amplified by a panel of V_H*back*/J_H *for* or Vκ*back*/Jκ*for* family-specific oligonucleotide primers [11]. The products were subcloned into pCR II.1 by use of the TA cloning system (InVitrogen). The recombinant plasmids containing identical DNA insert lengths were sequenced using Cy5 Autocycle Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) on a Pharmacia Biotech ALFexpress Automated DNA Sequencer. The sequences of several clones were compared by use of 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 Centre, Cambridge, UK).

Vaccine production

Hybrid cells that secreted immunoglobulin with the type of heavy and light chains corresponding to the tumour were expanded and finally cultured in a high-cell density system (miniPERM, Heraeus, Hanau, Germany) for the production of the idiotypic protein. Both tumour idiotypic proteins were pre-purified in HighTrap-IgM columns (Pharmacia Biotech). The absorbed material was eluted with 60% ethyleneglycol and further purified either by ionic-exchange chromatography (MONO-Q, Pharmacia) in case of clone 1 or by affinity chromatography (rProtL, ActiGen, Cambridge, UK) for clone 2. The purity of both idiotypic proteins was determined by SDS-PAGE and agarose-gel electrophoresis. Equivalent volumes of each idiotypic preparation (at 1 mg/ml) were mixed and added (1:1 vol.) to a solution of 1 mg/ml of KLH (Calbiochem, San Diego, Calif., USA) in PBS buffer. Glutaraldehyde was added for a final concentration of 0.1%, and the mixture was rotated for 4 h at room temperature. The reaction was quenched with 1 *M* glycine, and the preparation was then extensively dialysed against physiological saline. The final product was tested for sterility and general safety before use.

The immunological adjuvant SAF-1 was prepared as described by Allison and Byars [2]. Briefly, a mixture of Pluronic L-121 (BASF Corporation, Parsippany, N.J., USA), Squalane and Tween 20 (both from Sigma-Aldrich, St. Louis, Mo., USA) in PBS was homogenised in a Polytron machine and filtered through a 0.22 μm Corning filter. The patient received a series of five subcutaneous immunisations each consisting of 0.5 mg of the idiotypic immunogen mixed emulsified in SAF-1 adjuvant. Vaccines were administrated at day 0, and then 2, 6, 10 and 14 weeks later.

Humoral response

The presence of anti-idiotype antibodies in the patient's serum was studied by a four-layer enzyme-linked immunosorbent assay in which plates were first coated with a mouse monoclonal antibody (DA4–4, ATCC, HB-57) anti-human IgM. Tumour IgM, κp proteins or an unrelated IgM, κp captured by the mouse monoclonal antibody, were exposed to serially diluted pre-immune and post-immune patient's sera. The binding of anti-idiotypic antibodies was detected by horseradish peroxidase-goat anti-human IgG antibodies. KLH-coated wells were also tested for binding of anti-carrier antibodies. For clarity, only one dilution (1:81 for anti-idiotypic and 1:1,000 for KLH) is shown at each time point.

Results

Cytofluorometric analysis of cell suspensions obtained from LN cells showed an homogeneous peak of IgM, κ^+ cells (70%). Other surface markers studied were CD19 (60%), λ chain (4%) and IgG (4%). Fusion of LN cells with K6H6/B5 yielded 49% of wells with growing hybrids. As expected, most of the hybrid cells (96%) secreted an IgM κ-light chain.

The identity of the Ig derived from the somatic hybrids and tumour cells was studied by sequencing the gene segments VHDJ/VLJ. Samples of cDNA prepared from LN cells were amplified with V *back*/J_H *for* primers and cloned. The analysis of 15 sequences from independent bacterial colonies revealed the existence of two consensus sequences (Fig. 1), which included 12 and three clones, respectively. Searching for homologous germ-line sequences showed that the two tumour-derived sequences belonged to different members of the VH4 family (A: V4–61, B: V4–39). They also differed with regard to the D (A: D2–21, B: DN1) and J (A: JH6b, B: JH4b) gene segments. Moreover, consensus A showed only two nucleotide changes with respect to the germ-line V gene whereas consensus B was extensively mutated (21 nucleotide changes and one deletion of three nucleotides in FR2) compared with its germ-line V gene counterpart (Fig. 1).

Surprisingly, the initial analysis of the VH region used by two different hybridomas that were initially selected due to their high level of Ig secretion revealed that they matched the minor B consensus. This fact prompted us to study the VH used by a more ample representation of the generated hybridomas. The sequence analysis of more hybridomas revealed that consensus A and B were readily found in different hybridomas. The ratio of hybridomas using each of the two VH gene segments was 1:1.

In order to confirm the biclonality of the tumour sample, we also investigated the sequence of the Vκ light chain of the tumour cells and different hybridomas and

A

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           1                                   10                                      20
VH-Id1     CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCA CAG ACC CTG TCC CTC ACC TGC ACT GTC
V4-61      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                 30 _____________CDR1_________              40
VH-Id1     TCT GGT GGC TCC ATC AGC AGT GGT AGT TAC TAC TGG AGC TGG ATC CGG CAG CCC GCC GGA AAG GGA CTG GAG
V4-61      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G --- --- --- ---
                       ____________________________CDR2______________________________          70
VH-Id1     TGG ATT GGG CGT ATC TAT ACC AGT GGG AGC ACC AAC TAC AAC CCC TCC CTC AAG AGT CGA GTC ACC ATA TCA
V4-61      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                               80                                          90
VH-Id1     GTA GAC ACG TCC AAG AAC CAG TTC TCC CTG AAG CTG AGC TCT GTG ACC GCC GCA GAC ACG GCC GTG TAT TAC
V4-61      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

VH-Id1     TGT GCG AGC
V4-61      --- --- --A

DH/JH-Id1    TGTGCGAGCGCATATTGTGGTGGTGACTGCTATTTACCCTACTACTACGGTATGGACGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA
D2-21    113 ________A.........................CC
JH6b                     243 ______________________________.....TA................................G
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B

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                                               10                                      20
VH-Id2     ... ... .AG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC ACT GTC
V4-39      CAG CTG C-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                 30 ____________CDR1___________              40
VH-Id2     TCT GGT GCC TCC ATC ATC AGT AAT AAT TAC TAC TGG GGC TGG CTC CGC GA. ..C CCA GGG AAG GGA CTG GAG
V4-39      --- --- -G- --- --- -G- --- -G- -G- --- --- --- --- --- A-- --- C-G CC- --- --- --- --G --- ---
                       ____________________________CDR2______________________________          70
VH-Id2     TGG ATT GGG AGT ATC TCT TCT AGT GAG AGC ACC TTT TAC AAC CCA TCC CTC AAG AGT CGA ATC ACC ATA TCT
V4-39      --- --- --- --- --- -A- -A- --- -G- --- --- -AC --- --- --G --- --- --- --- --- G-- --- --- --C
                                               80                                          90
VH-Id2     GTA GCC ACG TCC AAG AGT CAG TTC TCC CTG ATA CTG AGC TCT GTG ACC GCC GCA GAC ACG GCT GTG TAT TAT
V4-39      --- -A- --- --- --- -AC --- --- --- --- -AG --- --- --- --- --- --- --- --- --- --- --- --- --C

VH-Id2      TGT GCG AGA
V4-39       --- --- ---

DH/JH-Id2        TGTGCGAGACAAGACCATACCAGTACCTGTTACTGCTTTGATACGTGGGGCCAGGGAACCCTGGTCACC
DN1         42   ____________.GGT...G...C.G...G...
JH4b       145   ______________________________...A......CTAC......................GTCTCCTCAG
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Fig. 1A, B Nucleotide sequences of the VHDJH gene segments used by **A** clone 1 and **B** clone 2. The *bottom sequence* represents that of the closest reported germline VHDJH gene segments. CDR regions are shown. Sequences homologous with the primer are *underlined*

found that two unrelated sequences were also present. The analysis of the Vκ/Jκ genes showed that two different sequences of germ-line origin were present in the neoplastic cells (**A**: DPK9/O12-Jκ2, **B**: DPK1/O18-Jκ4). These sequences were almost identical to their corresponding germinal genes (Fig. 2). All of the V4–61 hybridomas expressed the Vκ DPK9/O12 gene (clone 1), whereas a V4–39-DPK1/O18 pairing (clone 2) was found in the rest of the hybridomas analysed, confirming the presence of two unrelated tumour clones.

This patient was enrolled in the protocol of active immunotherapy developed at our centre. The biclonal origin of his neoplasia implicated the administration of the two idiotypic proteins present in the biclonal tumour. The idiotypic material secreted by each type of hybridoma was isolated from the spent medium of high-density cell cultures, mixed and conjugated to KLH. The purity of the final products is illustrated in Fig. 3, lanes 1 and 2. The idiotypic mixture with KLH (lane 5) did not show the presence of free material after coupling with glutaraldehyde (lane 6). The patient received five doses of subcutaneous injections of idiotypic protein-KLH conjugate emulsified in SAF. Local reactions consisted of mild erythema and induration of the skin, whereas general reactions were minimal (discomfort and febricula). Laboratory studies, including tests for anti-nuclear and anti-smooth muscle antibodies or rheumatoid factor, were negative.

The humoral response was screened by analysis of pre-immune and immune serum samples collected at various times. Increases in the antibody binding the autologous idiotype (and KLH) were detected after the second immunisation, and the levels of binding rose gradually, until they reached a plateau (Fig. 4) that was maintained for at least 6 months (not shown). The kinetics of anti-idiotypic antibodies directed against both

A

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             1                                            10                                                  20
VK-Id1     GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG
DPK9       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                   CDR1                                                 40
VK-Id1     GCA AGT CAG AGC ATT AGC AAC TAT TTA AAT TGG TAT CAG CAG AAC CCA GGG AAA GCC CCT AAG CTC CTG ATC
DPK9       --- --- --- --- --- --- -G- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- ---
                                   CDR2                             60                                    70
VK-Id1     TAT GCT GCA TCC AGT TTG CAA AGT GGG GTC CCA TCA AGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT
DPK9       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                  80                                      CDR3
VK-Id1     CTC ACC ATC AGC AGT CTG CAA CCT GAA GAT TTT GCA ACT TAC TAC TGT CAA CAG AGT TAC AGT ACC CCT
DPK9       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---


JK-Id1       TGTCAACAGAGTTACAGTACCCCTCAAACTTTTGGCCAGGGGACCAAGGTGGAGATCAAACGA
Jκ2     150  ______________________TGT.C....................C............
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B

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             1                                            10                                                  20
VK-Id2     GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CAG
DPK1       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                  CDR1                                                  40
VK-Id2     GCG AGT CAG GAC ATT AGC AAC TAT TTA AAT TGG TAT CAG CAG AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
DPK1       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                  CDR2                              60                                    70
VK-Id2     TAC GAT GCA TCC AAT TTG GAA ACA GGG GTC CCA TCA AGG TTC AGT GGA AGT GGA TCT GGG ACA GAT TTT ACT
DPK1       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                                                  80                                  CDR3
VK-Id2     TTC ACC ATC AGC AGC CTG CAG CCT GAA GAT ATT GCA ACA TAT TAC TGT CAA CAG TAT GAT AAT CTC CCC
DPK1       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T

VK-Id2        TGTCAACAGTATGATAATCTCCCCCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGT
Jκ4     181  ________________________G...................................
```

Fig. 2A, B Nucleotide sequences of the VKJK gene segments used by **A** clone 1 and **B** clone 2. The *bottom sequence* represents that of the closest reported germline VKJK gene. CDR regions are shown. Sequences homologous with the primer are *underlined*

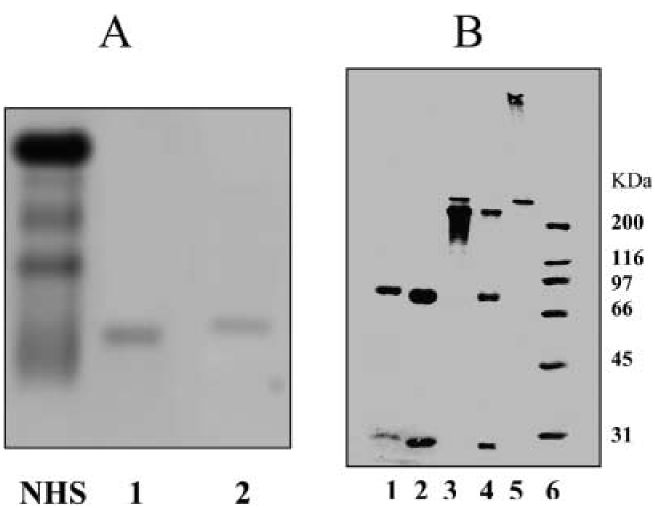

Fig. 3 **A** Agarose electrophoresis and **B** reducing 10% SDS-PAGE of the purified idiotypic proteins produced by clone 1 (*lane 1*) and clone 2 (*lane 2*). These two IgM proteins were mixed with KLH (*lane 4*) and linked by glutaraldehyde. No free Ig chains or KLH can be seen after coupling (*lane 5*). The high molecular weight of the conjugate prevented its entry into the gel. *Lane 3*: KLH, *lane 6*: molecular weight markers. *NHS* normal human serum

tumour idiotypes (Id1/Id2) were similar. Residual binding of hyper-immune serum to an isotype-matched unrelated idiotype (Id3) remained constant within the period of time studied (Fig. 4).

At the time of this report, the patient has completed the immunotherapy course and remains in complete remission, with no other treatment, 24 months after the last vaccine administration.

Discussion

In this report we show that it is feasible to rescue the two idiotypic proteins present in the tumour cells of a patient with a biclonal B-cell lymphoma for application of active immunotherapy. As far as we know, this is the first evidence of successful immunotherapy in complex neoplasias of this kind.

Different methods have been used for the routine diagnosis of clonality in B-cell lymphomas. In this context, immunohistochemistry, reactivity with anti-idiotypic antibodies and the detection of gene rearrangements or other low resolution methodologies [7, 14, 17] suffer from a number of limitations that have led to an underestimation of the frequency of biclonality in B-cell tumours. The development of hybridomas by fusion of tumour cells with an appropriate cell

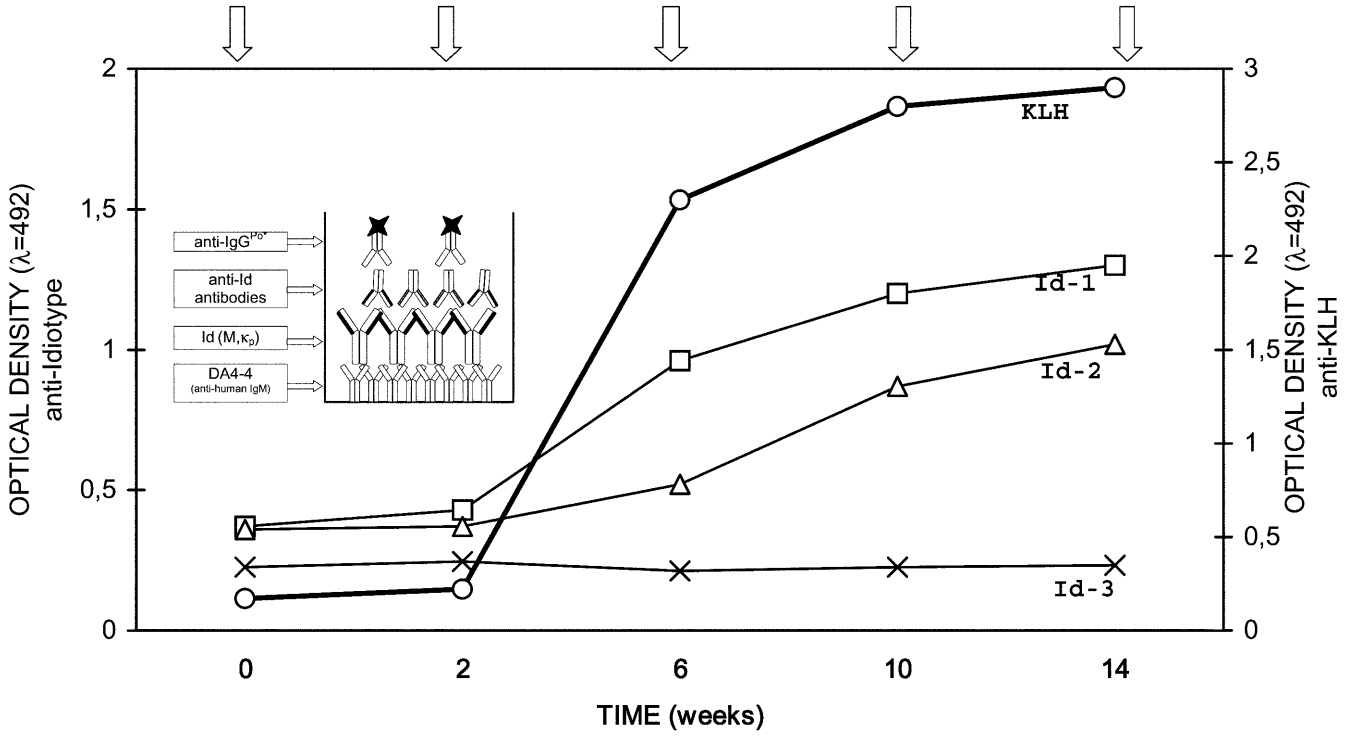

Fig. 4 Serum anti-idiotypic (Id-1: □; Id-2: △) and anti-KLH (○) antibody binding detected in pre-immune and post-immune serum samples. The patient was immunised at the indicated points (⇓). The reaction with a control unrelated IgM idiotype (Id-3: ×) is shown. The patient's sera were diluted 1:81 for anti-idiotypic and 1:1000 for anti-KLH assays. *Inset*: graphic representation of the enzyme-linked immunosorbent assay (ELISA) designed for anti-idiotypic antibody detection

line, permitted us to dissect the individual components of the tumour preparation. By combining the use of cloning and nucleotide sequencing of the rearranged Ig genes used by both the tumour and the hybridoma cells, we provide evidence of a truly biclonal B-cell lymphoma in a case in which the definition of biclonality is particularly difficult because both clones use identical light-chain isotypes. This is the first description, to our knowledge, of a biclonal B-cell tumour that was disclosed after analysis of the hybridomas obtained from tumour cells. It is of note that we did not find evidence of intra-clonal divergence within each of the two clones described, except for sporadic changes accounted for by the infidelity of the Taq polymerase reaction.

The different pattern of somatic mutation found in the two clones described in this work, where clone 1 VH and VK genes are almost unmutated whereas VH (but not VK) from clone 2 had acquired multiple mutations as regards their germ line counterparts, deserves a comment. Clone 2 contained 21 point mutations (eight in CDR1/2 that resulted in seven non-conservative and 1 conservative substitutions) and a deletion of three nucleotides in FR2. Somatic deletions are frequently found in expressed human V-region genes [8]. Previous reports indicate that the mutations that are found in many follicular centre lymphomas are acquired during physiological germinal centre (GC) reactions [6], and may affect differentially the heavy and light chains of the same tumour cell [16]. Moreover, the GC reaction can also result in receptor editing [10], leading to the expression of a new VH/VL combination. The development within a follicular cell tumour population of a distinct subpopulation endowed with a different potential of diversification has been reported [13]. However, we think that our findings are better explained by the existence of two independent neoplastic transformations, because a common origin with subsequent divergence of the two clonal populations from the original tumour would represent an unprecedented case of extreme receptor editing affecting both the kappa and heavy-chain variable genes. Further studies to characterise the development process affecting tumour cells could provide important clues to a better understanding of the biology of these non-monoclonal B-cell neoplasias.

In our patient, the efficient production of hybridomas originated from either clonal population of tumour cells, permitted us to accomplish the protocol of idiotypic vaccination. We decided to couple both idiotypes in the same immunological complex with the KLH carrier molecule in order to avoid differences in immunogenic properties that could be produced by using distinct Id-KLH conjugates. The titres of anti-idiotypic antibodies present in the patient's serum were similar for the two idiotypes and comparable to those observed in other patients with monoclonal tumours previously vaccinated by our group (data not shown). Our work highlights the importance of defining the clonality of tumour samples for developing protocols involving active idiotypic immunisation.

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References

1. Aarts WM, Bende RJ, Steenbergen EJ, Kluin PM, Ooms ECM, Pals ST, van Noesel CJM (2000) Variable heavy chain gene analysis of follicular lymphomas: correlation between heavy chain isotype expression and somatic mutation load. Blood 95: 2922
2. Allison AC, Byars NE (1986) An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity. J Immunol Methods 95: 157
3. Alvarez-Vallina L, Plaza A, Kreisler M, Cabrera R, Fernández MN, Díaz-Espada F (1995) Isolation of tumour-derived immunoglobulin-idiotype from peripheral blood mononuclear cells in a B-cell lymphoma patient with minimal disease. J Immunother 17: 194
4. Bahler DW, Levy R (1992) Clonal evolution of a follicular lymphoma: evidence for antigen selection. Proc Natl Acad Sci USA 89: 6770
5. Bendandi M, Gocke CD, Kobrin CB, Benko FA, Sternas LA, Pennington R, Watson TM, Reynolds CW, Gause BL, Duffey PL, Jaffe ES, Creekmore SP, Longo DL, Kwak LW (1999) Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nature Med. 5: 1171
6. Chapman CJ, Wright D, Feizi HP, Davis Z, Stevenson FK (1998) VH gene analysis of Burkitt's lymphoma in children from north-western Iran. Br J Haematol 103: 1116
7. Chen YT, Godwin TA, Mouradian JA (1991) Immunohistochemistry and gene rearrangement studies in the diagnosis of malignant lymphomas. A comparison of 152 cases. Hum Pathol 22: 1249
8. De Wildt RMT, Van Venrooij WJ, Winter G, Hoet RMA, Tomlinson IM (1999) Somatic insertions and deletions shape the human antibody repertoire. J Mol Biol 294: 701
9. Furuya T, Morgan R, Sandberg AA (1992) Cytogenetic biclonality in malignant haematological disorders. Cancer Genet Cytogenet 62: 25
10. Han S, Dillon SR, Zheng B, Shimoda M, Schlissel MS, Kelsoe G (1997) V(D)J Recombinase activity in a subset of germinal center B-lymphocytes. Science 278: 301
11. Hawkins R, Zhu D, Ovecka M, et al (1994) Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines. Blood 83: 3279
12. Kwak LW, Campbell MJ, Czerwinski D, Hart S, Miller R, Levy R (1992) Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumours. N Engl J Med 327: 1209
13. Matolcsy A, Schattner EJ, Knowles DM, Casali P (1999) Clonal evolution of B-cells in transformation from low- to high-grade lymphoma. Eur J Immunol 29: 1253
14. Simon M, Kind P, Kaudewitz P, et al (1998) Automated high-resolution polymerase chain reaction fragment analysis. Am J Pathol 152: 29
15. Sklar J, Cleary ML, Thielemans K, Gralow J, Warkne R, Levy R (1984) Biclonal B-cell lymphoma. N Engl J Med 311: 20
16. Stamatopoulos K, Kosmas C, Papadaki T, Pouliou E, Belessi C, Afendaki S, Anagnostou D, Loukopoulos D (1997) Follicular lymphoma immunoglobulin κ light chains are affected by the antigen selection process, but to a lesser degree than their partner heavy chains. Br J Haematol 96: 132
17. Wetter O, Brandhorst D, Breipohl W (1987) Differential immunocytological expression of a paraprotein-associated idiotype is possibly related to variant Ig molecules in plasmacytoma cells. Eur J Cancer Clin Oncol 23: 1633