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Active idiotypic vaccination in a patient with biclonal follicular lymphoma

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Abstract Specific immunological responses to the idiotype 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

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 VH (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 Biochemicals, 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 × 10⁶ LN cells or 0.5 × 10⁶ 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 Vγ back/JH for or Vλ back/JL 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 germ-line 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% ethylene glycol and further purified either by ion-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 1.0%, and the mixture was rotated at 4°C in the cold 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-idiotypic 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, κ protein or an unrelated IgM, κ 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/JH 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 bicolonality of the tumour sample, we also investigated the sequence of the Vκ light chain of the tumour cells and different hybridomas and