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Human monoclonal antibodies produced in transgenic BABκ,λ mice recognising idiotypic immunoglobulins of human lymphoma cells

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Abstract

Clonal idiotypic immunoglobulins of follicular lymphomas can be isolated by somatic fusion procedures. Idiotypic IgMs (Id-IgM) were isolated from two patients and used to immunise a strain of mice, deficient in mouse antibody production and engineered with yeast artificial chromosomes (YAC) containing fragments of the human immunoglobulin (Ig) μ/δ heavy chain and κ/λ light chain loci. Sequence analysis showed that hybridomas prepared from spleen cells of immunised mice expressed exclusively one of the six VH genes (VH1-2) present in the YAC transgene with different D/J rearrangements, and secrete fully human monoclonal antibodies (mAb) that recognised the tumour-specific IgM proteins. Further studies of the reactivity of the monoclonal anti-human Id-IgM antibodies revealed that they are specific for the individual protein of each patient and probably react with idiotypic determinants. In one case studied, the antibody recognised specifically the lymphoma cell expressing the corresponding idiotypic IgM and lysed those cells in the presence of complement. This is the first example of a human monoclonal antibody with such characteristics and may be of further use in the therapy of patients with B cell malignancies.

Keywords: Monoclonal Abs; Human anti-idiotypes; Transgenic mice; Immunotherapy lymphoma

1. Introduction

Tumours derived from clonal expansions of B lymphocytes are common in man, and can be identified by the expression of a particular tumor-especific antigen, the idiotype (Id) of the immunoglobulin molecule produced by the tumour clone. The induction of an immune attack targeted on idiotypic determinants can lead to destruction of tumour cells and this kind of immunotherapy has been applied in some kind of B cell tumours. In fact, the first use of monoclonal antibodies in human therapy began in 1982 when a patient with low grade lymphoma was infused with a murine anti-idiotypic antibody directed against the tumour Id (Miller et al., 1982). Despite the general excitement provoked by the initial success of the treatment, the limitations caused by the xenogenic nature of the murine mAb posed a great challenge to the general applicability of the method, that was later abandoned in favor of active immunotharapy strategies relying in the vaccination with immunogenic preparations of the patient's own tumour idiotypic immunoglobulin (Kwak et al., 1992; Bendandi et al., 1999; Barrios et al., 2002). However, the progression in the field of preparation of monoclonal material of human origin should lead to reconsider the passive immunotherapy treatment of certain B cell tumours. Although genetic and protein engineering techniques provided the tools to "humanise" the mouse sequences, without altering the original specificity of the antibodies (Jones et al., 1986; Riechmann et al., 1988) or even to obtain full-length human Fv fragments (Gilliland et al., 1996; Hayden et al., 1997), the development of the field has been disappointingly slow. A new method of obtaining human mAb has emerged since the availability of transgenic mice harbouring DNA fragments encoding human Igs. This method combines the specificity obtained by natural immunisation protocols with the well-proven hybridoma technology. One such transgenic mice is the BABκ,λ strain, which was developed by M. Bruggemann in Brabaham, UK, by crossing mice containing the human YAC transloci IgH/κ/λ, respectively, with mice containing non-functional endogenous IgH/κ loci (Kitamura and Rajewsky, 1992; Nicholson et al., 1999). Here, BABκ,λ mice have been used to prepare human mAb against idiotypic proteins obtained by somatic fusion of tumour samples from patients with B-cell low-grade lymphomas. The isolation

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and properties of two human monoclonal IgM antibodies with anti-idiotypic features are described in this paper. The mAbs bind specifically the idiotypic proteins and in one case studied recognised specifically and lysed in vitro the original tumour cells. The relevance of these findings in the context of the therapy of human lymphomas is discussed.

2. Material and methods

2.1. Translocus mouse strain or BAB5 mice

BABκ,λ mice were supplied by Marianne Brüggemann (The Babraham Institute, Babraham, Cambridge, UK). They were regularly tested for production of human IgM immunoglobulins by ELISA. The mice were kept in a pathogen-free environment. All experiments using mice were performed following the guidelines of Spanish regulations regarding the use of animals in scientific research (Real Decreto 223/1988).

2.2. Purification of human IgM monoclonal proteins and immunogen preparation

Monoclonal idiotypic (Id#) immunoglobulins were obtained by somatic fusion of tumour samples from six patients during the course of a Protocol of active immunotherapy against Id determinants of their follicular lymphomas carried out at our Centre. Details concerning the study design and Authorities Approval have been previously published (Barrios et al., 2002). Tumour cells were obtained from a diagnostic lymph node sample except in the case of patient #2, who presented with leukemic dissemination and blood lymphocytes were used as source of tumour cells. Ids#1–3 were IgMκ and Ids#4–6 IgGλ immunoglobulins. The Id# immunoglobulins secreted by hybrids obtained from each patient were purified by affinity chromatography and the Id#1 and Id#2 Igs were coupled to KLH (Calbiochem, San Diego, CA) as described (Barrios et al., 2002). These two latter preparations were used as immunogens to prepare anti-idiotypic antibodies by immunisation of transgenic mice bearing human μH and L chain genes. A schematic representation of the experimental procedures is depicted in Fig. 1.

Monoclonal IgMκ proteins were purified from the serum of patients with macroglobulinemia. The IgM concentration was adjusted to 1 mg/ml and 100 μl were fractionated in a Superosa 12 HR 10/30 column (Pharmacia Biotech, Uppsala, Sweden) in a FPLC device. The peak containing the IgM paraprotein was collected and used in further assays.

2.3. Generation of monoclonal antibodies

Groups of four mice, 2–4 months old, were immunised intraperitoneally with 100 μg of Id#1 or Id#2 IgMκ's coupled to KLH in Complete Freund's adjuvant (CFA, Calbiochem) or cholera toxin (Sigma Chemicals, St. Louis, MO) as adjuvants. The treated mice received two boost immunisations at 2 and 8 weeks with 50 μg of antigen in incomplete FA or cholera toxin. The titre of human anti-IgM and anti-KLH antibodies was studied by ELISA in serum samples taken 1 week after the last immunisation. The animals with the highest titre of anti-IgM antibodies received 2–3 weeks later an intravenous immunisation of 50 μg of antigen in saline solution and their spleen cells were fused with Sp/2 myeloma cells as described by Galfre and Milstein (1981). After hypoxanthine-aminopterine-thymidine

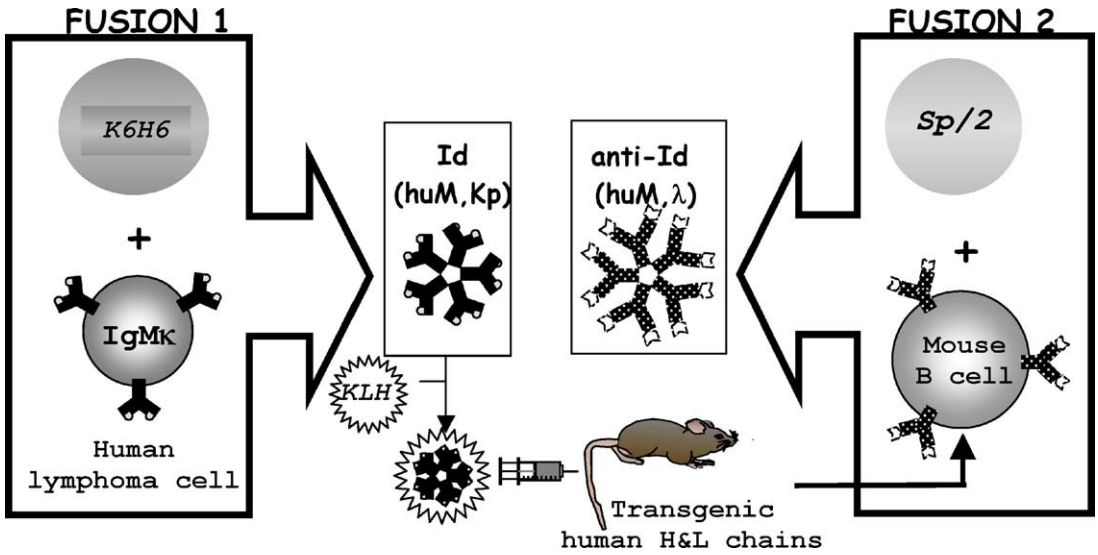

Fig. 1. A schematic view illustrating the preparation of lymphoma-specific human monoclonal antibodies. Tumour cells expressing a surface Id IgMκ are fused with the K6H6 heteromyeloma (Fusion 1) and the idiotypic immunoglobulin secreted by hybridoma cells is coupled to KLH and used to immunise BABκ,λ mice, transgenic for human heavy mu and light chains. The mouse spleen B cells are fused with Sp/2 (fusion 2) and the pentameric human IgMλ anti-Id antibody secreted by the corresponding hybridomas tested for binding to the immunising Id and to the Id-bearing lymphoma cells.

selection, hybridomas producing anti-IgM or anti-KLH were selected and cloned by limited dilution in HFCS (Roche Applied Science, Indianapolis, IN) medium.

2.4. Antibody assays

The reactivity of the human monoclonal antibodies produced by the mouse cells was measured by ELISA. IgMκ and IgGλ monoclonal Igs were captured in microplate wells coated with goat anti-human kappa and gamma chain antibodies, respectively (Sigma Chemicals). Blocked microplates were incubated with hybridoma supernatant samples. Binding of mAbs was assessed by developing with HRP-labelled goat anti-human lambda (in IgMκ wells) or mu chain antibodies (in IgGλ wells) (Sigma). The binding of IgMκ and IgGλ Igs was demonstrated by developing with HRP-anti-human mu or anti-human gamma antibodies, respectively. KLH was directly absorbed onto ELISA plates and mAb binding assessed by HRP anti-mu chain antibodies. The reaction was visualised with *O*-phenylenediamine (Sigma) at 492 nm. Titres of specific antibodies are expressed as the dilution giving half of the highest reading.

2.5. Electrophoretic and chromatographic analysis of human transgenic mice antibodies

To investigate the chain composition of the secreted material, 2 μl of culture supernatant were subjected to electrophoresis on a 1% SPE agarose gel (Beckman Instruments, Fullerton, CA) and transferred by pressure blotting (Aucouturier et al., 1987) onto PVDF membranes (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked with 3% fat-milk, treated with HRP-labelled goat anti-human mu, kappa or lambda chain specific antibodies (Sigma Chemicals) and the immunoreactive protein bands were visualised by chemiluminescence (Lumi-Light ^{Plus}; Roche Diagnostics, Mannheim, Germany).

The molecular size of the secreted human proteins was analysed by size-exclusion chromatography. The cell culture supernatant was concentrated 10-fold, and 100 μl were applied to a Superosa 12 HR 10/30 column. After filtration the anti-Id# reactivity and IgM content of each fraction was assayed by ELISA as described. The elution profile was related to standard proteins (human IgM, IgG, albumin) as indicated.

2.6. Sequence analysis of the hybridoma VH genes

Total RNA was extracted from 3×10^6 hybridoma cells (S.N.A.P., InVitrogen, Carlsbad, CA). cDNA synthesis was performed with AMV reverse transcriptase using an oligo (dT) primer (Promega, Madison, WI). cDNA was amplified using a panel of 5′-VH oligonucleotide primers specific for the members of the VH-subfamily present in the IgH YAC transloci (VH1, VH2, VH4 and VH6) and a 3′- primer complementary to a human CH1μ sequence (Word et al., 1989). The products were subcloned into pCR^{R}4-TOPO^{Rb} by the TOPO TA cloning system (InVitrogen, Carlsbab, CA) and the recombinant plasmids that contained the VDJ-CH1μ inserts were sequenced using the Cy5 Auto-Cycle Sequencing Kit (Pharmacia Biotech) on a Pharmacia Biotech ALFexpress Automated DNA Sequencer. The analysis of the sequences was undertaken using the MicroGenie Program (Beckman). Finally, the search for homologous germline sequences, the alignments, and the analysis of the junctions VDJ was carried out using the following web sites: http://imgt.cines.fr:8104, //www.mrc-cpe.cam.ac.uk and www.toulouse.inra.fr/multalin.html.

2.7. Flow cytometric analysis

Specific antibodies were tested by indirect immunofluorescence using a cell suspension obtained from tumour samples from patients #2–5. A total of 2×10^5 cells were incubated with hybridoma supernatants, washed and stained with a PE-conjugated mAb antibody anti-human lambda chain (Clone MC24-IC6, Serotec Ltd, Oxford, UK) (patients #2 and 3) or a FITC-conjugated mAb antibody anti-human mu chain (Clone M15/8, Serotec Ltd) (patients #4 and 5). The cells were analysed in an EPICS XL-MCL cytometer (Coulter, Hialeah, FL).

2.8. Complement-mediated cell lysis using the anti-IgM antibodies

The tumour cells from patient #2 were incubated with supernatant from hybridoma clones, followed by the addition of rabbit serum (BAG, Lich, Germany). The viability of the cells was assessed by contrast microscopy after a conventional Terasaki microlymphocytotoxicity assay.

3. Results

3.1. Production of human antibodies to human IgM

Transgenic mice, immunised with idiotypic human IgM coupled to KLH, produced antibodies to both the idiotypic protein and the carrier molecule. The titre of serum antibodies found in different animals ranged from 1/100 to 1/3000 for the KLH molecule and 1/20 to 1/80 for IgM. There were no differences in antibody titres when using cholera toxin or CFA/IFA as adjuvants (data not shown). Of 70 IgM secreting clones generated in two different productive fusions, 16 were found to bind the KLH carrier molecule and only four recognised the immunising idiotypic IgM. In order to discriminate the immunising from the produced mAb only a HRP human λ-chain reagent was used; therefore, it is possible that the real number of clones producing anti-Id antibodies could be higher. Two hybridomas producing IgMλ antibodies, designated 2D1 and 1E3, were stabilised by

Table 1
Reactivity of human mAbs against human Ig proteins measured by ELISA

		Id#1	Id#2	Id#3	Id#4	Id#5	Id#6	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
2D1	α-λ	2.20	0.09	0.12				0.07	0.09	0.14	0.12	0.14	0.14	0.12	0.08	0.22	0.10
1E3	α-λ	0.09	1.95	0.10				0.08	0.09	0.15	0.21	0.16	0.14	0.13	0.11	0.30	0.09
2D1	α-μ				0.04	0.04	0.05										
1E3	α-μ				0.03	0.04	0.05										
CM	α-μ	3.05	3.05	3.05				3.50	2.90	3.70	3.40	3.40	3.20	3.20	3.30	3.30	3.20
CM	α-λ				1.80	1.60	1.10										

Preparations of idiotypic lymphoma Igs (Id#1–3: IgMκ; Id#4–6: IgGλ) or IgMκ Igs from macroglobulinemic sera (M1–10) were absorbed onto wells coated with anti-human kappa (Id#1–3; M1–10) or anti gamma (Id#4–6) chain antibodies and incubated with 2D1 mAb, 1E3 mAb or culture medium (CM). Wells were developed with the indicated goat anti-human λ, μ or γ-peroxidase- labelled antibodies. Arithmetic means of absorbance readings at 492 nm of triplicate wells are indicated.

repeated cloning dilution and their specificity and properties further studied.

3.2. Reactivity of the anti-Ids antibodies

To test the specificity of the anti-Id antibodies, the hybrid supernatants were incubated in wells coated with a panel of monoclonal human immunoglobulins. Ids#1–6 were idiotypic Igs obtained from tumour cells of lymphoma patients, whereas IgMκ 1–10 were monoclonal paraproteins from macroglobulinemic sera. As can be seen in Table 1 the 2D1 and 1E3 mAb were exclusively directed against the immunising Id#1 or Id#2 idiotypic immunoglobulin, respectively. A further proof of the anti-Id specificity of the anti-Id mAbs is also presented in Table 1, where it is shown that each anti-Id mAb did not bind to any of five unrelated idiotypic immunoglobulins (IgM or IgG isotype) or to any of ten unrelated monoclonal IgMk proteins.

3.3. Presence of free light chains and polymeric assembly of the human anti-IgM antibodies

The production of an excess of free light chains is a common finding in mouse hybridomas. The presence of such free light chains in hybridoma supernatants was revealed by electrophoretic separation of the monoclonal components. As shown in Fig. 2A, the 2D1 hybridoma produced a free lambda chain in addition to the whole IgM molecule, whereas no free light chains were found in the 1E3 supernatant.

M_r analysis by size-exclusion chromatography of the 2D1 and 1E3 supernatants (Fig. 2B) revealed that anti-idiotypic activity was confined to the fractions containing polymeric IgM, which excludes any interference of the free light chain in the reactivity of the 2D1 antibody.

3.4. VH gene expression and sequences of anti-Id antibodies

Both the 2D1 and 1E3 hybridomas used the VH1-2∗02 gene segment, but distinct DJH rearrangements were found in the 2D1 (D1-1∗01/JH4∗02) and in the 1E3 hybridoma (D3-10∗01/JH6∗02). The sequence of the rearranged 2D1 VH gene was identical to the VH1-2∗02 germline gene, whereas the 1E3 VH gene sequence showed up to eight different nucleotide changes (Fig. 3). These changes resulted in only two amino acid replacements located in the framework regions (K13N; S84N) and in the CDR2 (N54I), respectively. In the 2D1 hybridoma, the recombination process involved the deletion of 3′ and 5′ D nucleotides, but

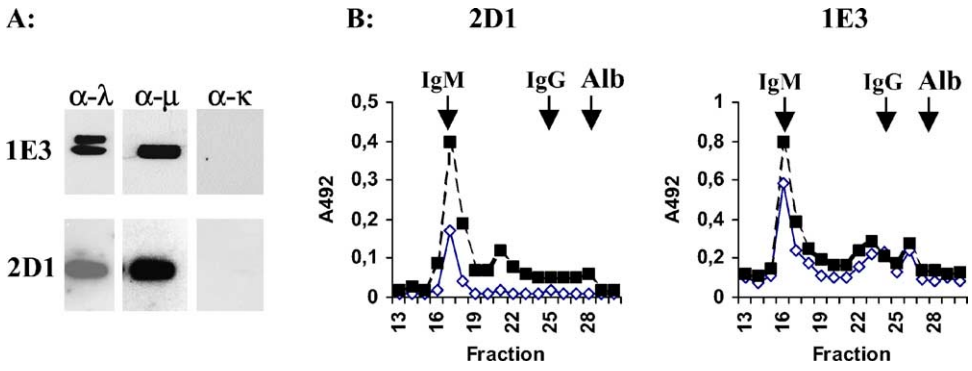

Fig. 2. Assembly of the human 2D1 and 1E3 mAb. (A) Hybridoma supernatants were fractionated by agarose gel electrophoresis and transferred to PVDF membranes. The Ig composition was studied by developing different lanes with peroxidase-labelled anti-human λ, μ or κ chain antibodies, followed by chemiluminiscence. (B) Gel filtration of the 2D1 and 1E3 mAb culture supernatants on a Superose 12 column. The eluted fractions were tested for IgM content (closed symbols) and anti-idiotypic activity (open symbols) against the corresponding idiotype by ELISA (A_{492}). The elution positions of IgM, IgG and Albumin are indicated.

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                ________FR1____________   ___CDR2___   ____________FR3________________
                        10                    54                    80
                  G   A   E   V   K   K    P   N   S    S   T   A   Y   M   E   L   S
VH1-2*02 GGG GCT GAG GTG AAG AAG  CCT AAC AGT  AGC ACA GCC TAC ATG GAG CTG AGC
2D1      ... ... ... ... ... ...  ... ... ...  ... ... ... ... ... ... ... ...
1E3      ... C.. ... ..t ..a ..C  ... .T. ...  ..t ... ... ... ... ... ... .A.

CDR3:                                                                      Length
           C   A   R   T   T   G   T          T   Y   F   D   Y   W
2D1      tgt gcg aga aca act gga act        acc tac ttt gac tac tgg       11

           C   A   R   S   F   G   D      Y   R   G   M   D   V   W
1E3      tgt gcg aga tcc ttc ggt gac    tac cgc ggt atg gac gtc tgg       12
```

Fig. 3. Sequences of heavy chain variable regions from 2D1 and 1E3 mAb antibodies are aligned with the germline gene VH1-2∗02 sequence. For brevity, only VH codons are shown at selected positions where nucleotides replacements occur. Dashes indicate nucleotide identity. Nucleotide changes leading to amino acid replacements are in uppercase letters. DH sequences are underlined. The D1-1∗01/JH4∗02 and D3-10∗01/JH6∗02 JH genes were rearranged in the hybridomas 2D1 and 1E3, respectively.

the JH 4∗02 gene was in germline configuration, whereas in the 1E3 hybridoma nucleotide deletions occurred in both the D and JH segments. For both hybridomas, the recombination process did not involve the use of nucleotides from the 3′ non-coding region of VH1-2∗02 and putative N or P nucleotide additions were not found at the V-D-J junctions. The 2D1 and 1E3 VDJ nucleotide sequences are available in the GenBank database under the accession numbers: AY335903 (2D1) and AY335904 (1E3).

3.5. Reactivity of the 1E3 anti-#Id2 antibody with donor tumoral cells

We have previously shown that the anti-IgM mAb recognised specifically monoclonal immunoglobulins obtained by somatic fusion from lymphoma cells. In order to validate the future use of the monoclonal antibodies in human therapy, it must be demonstrated that these mAb also recognise specifically the original tumour cells. To this aim, we used a PBM cell preparation from patient #2, which showed leukemic dissemination, and suspensions of lymph node tumour cells from patients #3–5. Tumour cells from patients #1 and 6 were not available at the time of the present study. Indirect staining with the 2D1 anti-Id#1 and 1E3 anti-Id#2 antibodies plus an appropriate secondary reagent (PE-labelled anti-λ or FITC-labelled anti-μ) showed that only the anti-Id#2 monoclonal antibody was able to bind to the tumour cells from patient #2 (Fig. 4A). The anti-Id#2 mAb did not recognise the tumour cells of patients #3–5. To test whether the mAb induce complement-mediated

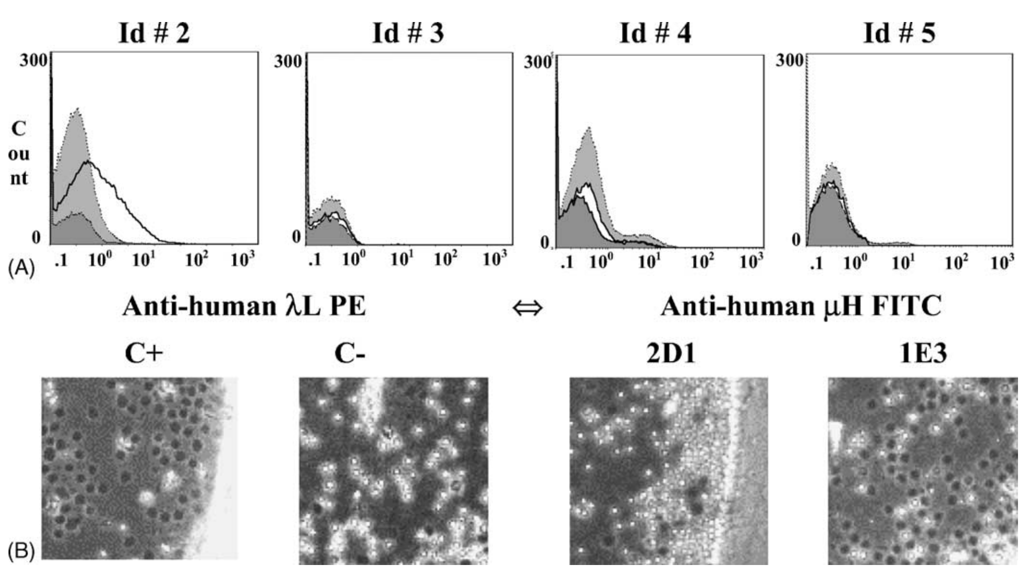

Fig. 4. Human mAb 1E3 recognise and lyse idiotype positive Id#2 lymphoma cells. (A) Flow cytometry analysis of Id#2–5 lymphoma cells incubated with the 2D1 anti-Id#1 or the 1E3 anti-Id#2 mAbs and stained with the indicated secondary reagents. The white and grey histograms represent cells treated with the 1E3 and 2D1 mAb, respectively. Surface staining with isotype control are shown by silver histograms. (B) Complement mediated cell lysis. Id#2 lymphoma cells were incubated with the 2D1 or 1E3 mAb plus rabbit complement and the dead cells were identified by eosin staining (black dots) in Terasaki plates. An anti-human HLA serum and culture medium served as positive (C+) and negative (C−) controls, respectively.

cell lysis, Id#2 cells were incubated with hybridoma supernatants and the extent of cell lysis was investigated by microscopic inspection in a Terasaki lymphotoxicity assay. As it is shown in Fig. 4B, the lymphoma cells were lysed in the presence of the 1E3 mAb and rabbit complement, whereas the non-binder 2D1 did not trigger cell lysis.

4. Discussion

The production of monoclonal antibodies of human origin is of great relevance for circumventing the problems that limit the use of rodent mAb in human therapeutics. Although recombinant DNA techniques offer the opportunity of obtaining "humanised" antibodies, a further advance is the development of transgenic mouse strains engineered to produce human antibodies. To generate monoclonal antibodies of the desired specificity in these transgenic strains, standard hybridoma technology can be easily adapted without the need for more sophisticated molecular technologies. This approach is particularly important in the treatment of B cell malignancies, where the tumour cells expresses a well-defined tumour specific antigen, the idiotype of the clonal immunoglobulin produced by the tumour cell. We took advantage of our previous work on idiotypic vaccination with tumour-derived idiotypes (Barrios et al., 2002) to address the issue of preparation of human monoclonal antibodies directed against idiotypic determinants present in follicular lymphoma cells.

The production of human monoclonal antibodies to human antigens in transgenic BABκ,λ mice was recently reported by our group (Magadan et al., 2002). The BABκ,λ mouse strain produces human IgM antibodies with a lambda/kp ratio of 2/1. Unlike the BABκ from which they derive, BABκ,λ mice produce only marginal amounts of the endogenous mouse lambda chain, making unlikely the presence of quimeric antibodies. All hybridomas derived from strains containing the 240-kb yH1 transloci used exclusively the VH1-2 gene segment. This does not prevent them from expressing a wide repertoire of specificities (Nicholson et al., 1999).

Murine anti-Id antibodies were initially used in the treatment of B cell malignancies, but were later abandoned for more unspecific non-clonal reagents. In this report, the preparation of human anti-idiotypic antibodies in BABκ,λ mice is addressed. For the transgenic BABκ,λ mouse, the repertoire encoded for by the human Ig transgenes must be shaped to become tolerant in a microenvironment dominated by mouse proteins. Additionally, the presence of circulating human IgM proteins must induce tolerance to isotypic determinants present in human μ, κ and λ Ig chains. Thus, anti-human IgM antibodies prepared in that strain of mouse must recognize determinants localised in the V region of the immunising IgM molecule (or allotypic determinants in the kappa chains).

Fully human monoclonal antibodies binding two Id immunoglobulins expressed by the cells of two patients with follicular lymphoma were obtained. These two Id immunoglobulins were coded by the germline gene segments VH4-39∗01/Vκ1–33 (Id#1) and VH3-48∗03/Vκ1–39 (Id#2) (Barrios et al., 2001; Barrios et al., 2002), none of which were contained in the IgH YAC transloci present in the BABκ,λ mouse genome (Nicholson et al., 1999). Therefore, although it may be possible that the anti-Id antibodies recognise epitopes located in framework regions, the fact the each anti-Id antibody binds exclusively to the immunising Id and not to any of the 14 different monoclonal proteins tested suggested that the monoclonal reagents have a truly anti-idiotypic nature. Although the combinatorial antibody phage display system has provided human anti-idiotypic Fv material (Fischer et al., 1999), the monoclonal antibodies reported here are to the best of our knowledge the first fully human anti-idiotypic antibodies obtained by hybridoma technology.

Our results show that, as happens in a physiological autologous setting, the anti-human anti-Id response was not suppressed in the xenogenic mouse host. However, and contrary to what is usually encountered in normal anti-self idiotypic antibody responses (Meek and Kapra, 1990), no evidence of somatic hypermutation in the V regions of the 2D1 and 1E 3 mAb was found. The gene segments coding for the anti-Id antibodies described here contain very few or no non-silent mutations in the V regions and no additions of N and P nucleotides. Therefore, their specificity must be mainly conferred by D and J rearrangements. All these features are characteristic of natural antibodies (Sanz et al., 1989), suggesting an internal autoreactivity in the naive human repertoire.

As the idiotypic determinants constitute a well defined, clonally-specific tumour antigen, these fully human anti-Id monoclonal antibodies may prove effective in the treatment of B cell malignancies. The binding of mAb to tumour cells in vivo can mediate tumour destruction either by initiating the complement attack or by recruiting citotoxic or phagocitic cells. The anti-Id human IgM antibodies are secreted as polymeric molecules and can specifically bind and lyse in the presence of rabbit complement the tumour cell from which the immunoglobulin idiotype was obtained. However, the tumour cells of patient #2 were not lysed by the 1E3 mAb in the presence of human complement (data not shown). This resistance to lysis induced by mAbs in the presence of human complement is a property of some tumours in vitro and both the amount of the targeted antigen (Golay et al., 2001) and the level of inhibitors of the C3 convertase in the cell surface (Fishelson et al., 2003) are important factors determining the behaviour of the tumour cell. Nevertheless, there is no clear relationship between the level of complement-regulatory protein expression of tumour cells and the clinical response to in vivo treatment with mAb (Bannerji et al., 2003). Additional mechanisms of tumour destruction in vivo could be provided by the Fcμ

receptors present in fagocitic (Shibuya et al., 2000) and NK cells (Pricop et al., 1991). The central role of Fc receptors in the therapeutic efficacy of human McAbs has being recently shown (Cartron et al., 2002), although the effectiveness of IgM isotypes in this setting remains to be established.

It should be stressed that the need to produce an anti-idiotypic antibody for each patient severely limits the general application of the method. Although in this work we have not found evidence of crossreactivity, it is possible that the study of a larger panel of anti-idiotypic mAbs reveals idiotope-sharing between different idiotypic proteins (Miller et al., 1989) and individual reagents could be used in the treatment of several patients. The method of producing anti-Id human mAb reagents described here has some limitations, mainly due to the uncertainties imposed by the need for obtaining two monoclonal products. On the other hand, hybridomas producing mAb binding cell-surface antigens are easily obtained from BABκ,λ mice immunized with human cells (Magadan et al., 2002). Thus, a possible way to circumvent these limitations may lie in the production of the required human monoclonal reagents specific of individual idiotypes by the immunization of the Transgenic mice strain with tumoral B cells. If this method proves to be effective, the earlier and quickly abandoned attempts to induce long-lasting remission of B cell lymphomas by passive immunization with specific reagents can be revitalized.

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