Monoclonal Anti-Light Chain Idiotype as a Tumor-Specific Probe for Human Neoplastic B Lymphocytes


Tumor cells from patients with B cell neoplasms often secrete small amounts of free monoclonal light chains that can be found in the urine. Such tumor-derived light chains of the λ type from a patient with typical chronic lymphocytic leukemia have been used to raise mouse monoclonal antibodies (MoAbs). A hybridoma-secreting antibody that recognized the idiotype λ chain but not normal λ chains by a preliminary screen but which also reacted with idiotypic IgM from the patient's tumor cells was selected. This MoAb in fact recognized 1 in 20 x 10^10 molecules of pooled normal λ chains, thus establishing its specificity for a private idiotype determinant. It failed to give a detectable reaction with normal IgM, normal serum, or a panel of IgM paraproteins. The antibody bound to the patient's neoplastic B cells but not to normal tonsillar cells. The site of binding of the antibody to idiotypic IgM is clearly separate from that of another MoAb specific for idiotype determinants on heavy plus light chains, since the two showed additive binding curves. The determinant also appeared to be less available in dimeric λ chains than in monomeric λ chains or in idiotypic IgM. Antibodies to idiotype determinants on light chains show some technical advantages and should be useful for monitoring and possibly treating B cell tumors, either alone or together with the more conventional anti-idiotype antibodies that usually recognize the heavy and light chain combination.

THE IDIOTYPIC determinants of the surface immunglobulin (Ig) of neoplastic B lymphocytes represent a molecularly defined tumor-associated antigen. Antibodies specific for these determinants have been used for treatment of human lymphoma with some indication of success, although difficulties remain. Monitoring of disease with anti-idiotype, however, either by identification of tumor cells or by assay of free serum idiotype, presents few problems.

Nevertheless, difficulty in raising such antibodies has prevented widespread use by clinicians, since idiotypic Ig for immunization must first be obtained from the tumor cells, perhaps from a small biopsy, either by enzyme digestion or by rescue hybridization, neither of which is a routine procedure. The use of impure immunogen such as tumor cells or membrane preparations, although possible, yields only a small proportion of anti-idiotype hybridomas from which to select. The use of an alternative source of immunogen, such as the monoclonal light chain derived from tumor cells and found in urine, therefore could represent a simplification of the approach.

In a study of patients with non-Hodgkin's lymphoma, 23/24 patients had tumor cells that secreted free monotypic light chains. In a separate investigation of random urine samples from patients with B cell disease, about 50% showed monoclonal light chain by sensitive isoelectric focusing. Presumably the level of the urinary light chain depends partly on tumor load. Such light chains can be prepared by simple immunosorption methods and have been used previously to raise polyclonal antisera for four patients with chronic lymphocytic leukemia (CLL). One notable finding was that these four antisera, rendered specific for idiotype by absorption, recognized the cell surface IgM on tumor cells from the original patients. The finding was unexpected, since in the reciprocal situation anti-idiotype raised against combined (heavy plus light chain) idiotype usually does not recognize free light chain.

To analyze more critically whether the same antibody could recognize both free and combined light chain idiotype, we have raised a monoclonal antibody (MoAb) with this dual recognition. We have also compared it with a conventional anti-idiotype antibody raised against idiotypic IgM from the same patient.

MATERIALS AND METHODS

Patient and Cell Preparation

The male patient LP, aged 50 years, presented with typical CLL with a WBC of 50 x 10^9/L consisting of >95% neoplastic B cells that expressed IgM and IgD, both with the λ light chain, on the surface with no detectable intracellular Ig. A bone marrow sample also showed heavy infiltration with tumor cells, >90% expressing IgM, D, light chains. Serum immunoglobulins were at the low end of the normal range, and no paraprotein was detected on routine electrophoresis. Cells were prepared from peripheral blood and were usually frozen in a controlled cell freezer unit for subsequent investigation. Urine was collected as 24-hour samples directly into bottles containing 5 mL toluene as preservative.

Urinary Light Chain

A sample of concentrated (x 100) urine was analyzed by isoelectric focusing (IEF) with immunofixation as described. Preparation of the identified λ chain was by sequential immunosorption. Briefly, urine (500 mL) was passed through a series of 10-mL columns consisting of Sepharose 4B-CL linked to: (1) normal sheep IgG to remove nonspecific adherent material; (2) sheep anti-K to remove K light chains; and (3) sheep anti-λ to bind the λ chains. Elution of the last column with 0.5 mol/L NH4OH-1.0 mol/L KSCN, followed by dialysis into cold 0.1 mol/L Tris-HCl-l mol/L NaCl to 0.2% NaN3, pH 8, isolated the relevant light chain. Any sheep protein detached from the immunosorbent during elution was removed by passage through an immunosorbent of rabbit antiship IgG (5 mL). The purity of the final product was examined by IEF, and amounts were

From the Lymphoma Research Unit, Tenovus Research Laboratory, General Hospital, Southampton, England.

Submitted July 1, 1986; accepted October 17, 1986.

Supported by Tenovus of Cardiff, the Cancer Research Campaign, and the Leukaemia Research Fund, England. We also thank NATO for a collaborative grant.

Address reprint requests to Dr FK Stevenson, Lymphoma Research Unit, Tenovus Research Laboratory, General Hospital, Southampton, England.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/6903-0041$3.00/0

measured at each stage by enzyme-linked immunosorbent assay (ELISA). Separation of disulfide-bonded dimeric from monomeric \( \lambda \) chain was by passage through a column of Sephadex G-100 (Pharmacia) equilibrated with propionic acid (1 mol/L). The separated peaks were dialyzed into acetate buffer (4 mmol/L, pH 5.4) and stored at \(-20^\circ \text{C}\). To obtain normal \( \lambda \) chains, Ig\( \alpha\) was first prepared from normal human Ig\( \alpha\) by immunosorption. This Ig\( \alpha\) was then reduced and alkylated and the monomeric \( \lambda \) chains separated in propionic acid as above.

**Monoclonal Anti-idiotypes**

Anti-\( \lambda \) chain idiotype (anti-Id \( \lambda \)). The isolated urinary \( \lambda \) chain was used to immunize a BALB/c mouse with a primary subcutaneous (SC) injection of 100 \( \mu \)g in Freund's complete adjuvant (CFA), followed by similar booster injections after 5, 7, and 9 weeks. The final injection was of 100 \( \mu \)g aqueous antigen intravenously (IV) three days before removal of the spleen for hybridization of the cells with the mouse myeloma NS-1 (P3-NS-1/1-Ag 4.1). The procedures for growing colonies and cloning were as described, selection being based on reactivity of supernatants in the ELISA system with idiotypic \( \lambda \) chain (Id\( \lambda\)), Ig\( M\)Id, and irrelevant light chains. To produce larger amounts, 10\( ^6 \) hybridoma cells were injected intraperitoneally (IP) into Pristane-primed BALB/c mice, and antibody was purified from ascitic fluid by precipitation with ammonium sulfate followed by separation on a column of diethyl aminoethyl (DEAE)-trisacryl in phosphate buffer (5 mmol/L, pH 8) with a linear gradient to 50 mmol/L. The major peak was collected and its purity checked by electrophoresis.

Anticom pared idiotypic (anti-Id \( \mu + \lambda \)). To obtain Ig\( M\)Id for immunization, a "rescue" hybridization was carried out by fusing tumor cells from the blood of patient LP with the mouse myeloma NS-O, a subline that synthesizes no mouse \( \kappa \) chain. Colonies producing Ig\( M\)Id were cloned by limiting dilution and supernatants with no complications due to different coating efficiencies or to possible structural distortions occurring during binding to a solid phase.

**Immunofluorescence**

Testing of anti-idiotypes on the patient's dispersed tumor cells was by flow cytometry on the FACS III (Becton Dickinson, Mountain View, CA). For inhibition studies, normal human serum (50 \( \mu \)L) was added to culture supernatants (50 \( \mu \)L) or to purified antibody at \(-20 \mu \text{g/mL}\), before adding to the cells. Bound antibody was detected with fluorescent rabbit antimouse Ig.

**Tissue Sections**

Frozen tissue sections were used to assess reactivity of the anti-Id\( \lambda\) with normal tonsil and lymph nodes from nine patients with lymphoma. Cryostat sections (5 \( \mu \)m) were air-dried and stored at \(-70^\circ \text{C}\) until required. Immediately before staining the sections were fixed in acetone for ten minutes and then stained by a two-layer immunoperoxidase technique, using HRP-rabbit antimouse Ig as the second layer and developing reaction product with diaminobenzidine tetrahydrochloride.

**RESULTS**

**Production of \( \lambda \) Id by Tumor Cells**

Neoplastic B cells from LP secreted in vitro free \( \lambda \) light chain (20 \( \mu \)g/h) and Ig\( M\) (7.2 \( \mu \)g/h), values in the range of those found previously for patients with CLL. The levels of light chain (\( \kappa \) and \( \lambda \)) in a typical 24-hour urine sample from LP were 0.3 (\( \kappa \)) and 6.8 (\( \lambda \)) mg per 24 hours, which is below the detection level of most routine methods and requires sensitive IEF and immunofixation or other special techniques for identification. Identification of the monoclonal \( \lambda \) chain for LP by this technique has been reported. After purification of the urinary Id\( \lambda\) and passage through G-100 in 1 mol/L propionic acid, the disulfide-bonded dimer accounted for about 20% of total \( \lambda\).

**Monoclonal Anti-Id (\( \lambda \))**

In the first screen of selected hybridoma supernatants, seven clones were found to recognize Id\( \lambda\) and not pooled Id\( \kappa\).

**Inhibition ELISA to Quantitate Relative Binding**

This was established to measure the relative abilities of different \( \lambda \)-containing molecules to bind to the MoAb-Id\( \lambda\)(A) and also to find the proportion of Id\( \lambda\) among normal \( \lambda \) chains. First Id\( \lambda\) was bound directly to the microtitre plate at 100 ng/mL. Monoclonal antibody was then added and bound mouse Ig detected with horse-radish peroxidase (HRP)-labeled rabbit antimouse Ig (Nordic Labs, Maidenhead, England). Binding to Ig\( M\) was investigated by the indirect procedure: rabbit antihuman \( \kappa \) chain (10 \( \mu \)g/mL) was coated onto the plate and Ig\( M\) preparations, and either "rescue" hybridoma supernatants or macroglobulinemic sera were added, followed by MoAb as above.

**Binding ELISA to Assess Specificity**

A preliminary assessment of specificity was carried out using Id\( \lambda\) or a pool of three \( \lambda \) Bence-Jones proteins coated directly onto a microtitre plate at 100 ng/mL. Monoclonal antibody was then added and bound mouse Ig detected with horseradish peroxidase (HRP)-labeled rabbit antimouse Ig. (A) 1: Anti-human \( \kappa \) chain (10 \( \mu \)g/mL); 2: anti-human \( \lambda \) chain constant region (10 \( \mu \)g/mL); 3: antibody against heavy plus light chain idiotype (anti-Id \( \mu + \lambda \)) (10 \( \mu \)g/mL). (B) 1: Anti-human \( \kappa \) chain (10 \( \mu \)g/mL); 2: anti-human \( \lambda \) chain constant region (10 \( \mu \)g/mL); 3: antibody against \( \lambda \) chain idiotype (anti-Id \( \lambda \)) (24 \( \mu \)g/mL); 4: anti-Id (\( \lambda \)) plus normal human serum (50%).
chains of these, three also recognized IgMId and not normal IgM, and the most vigorous of these was selected for expansion. Further tests of specificity showed the antibody to be unreactive with rescued IgM preparations (3 IgMλ) or 23 sera from macroglobulinemic patients (10 IgMκ and 13 IgMλ). The subclass of the antibody was IgG1.

Immunofluorescence showed good reactivity with tumor cells from LP, which was not affected by the presence of normal human serum (50%) (Fig 1). The antibody did not react with frozen sections of normal tonsil nor with nine lymph node specimens from patients with B cell lymphoma; antibodies to Ig constant region reacted normally with these sections.

Monoclonal Anti-Id (μ + λ)

This antibody was selected by the usual criteria for anti-idiotypic considered for therapy, ie, it recognized IgMId but not normal IgM by ELISA, and its binding to tumor cells from LP (Fig 1) was not inhibited by normal serum (50%). This antibody did not bind to λId by ELISA, and its binding to IgMId was unaffected by λId up to a concentration of 50 μg/mL.

Nature of Determinant Recognized by Anti-Id(λ)

Monomeric and dimeric λ Id. It was of interest to determine the reactivity of the monoclonal anti-Id (λ) with monomeric or dimeric λId to see if the relevant determinant was more exposed on the monomer. Disulfide-bonded dimer and monomer were first assayed by ELISA using anti-λ constant region antibody on the plate and detecting bound λ with HRP-anti-λ. Results are shown in Fig 2 where it is clear that at saturation, monomer gives half the binding of dimer at equivalent concentrations. This is consistent with equal molar binding to the plate, with each bound dimer offering twice as many determinants as monomer.

Comparison of the ability of the monomer and dimer to bind to the anti-Id (λ) was made by the inhibition ELISA (Fig 3). It was found that λId monomer was approximately 12x more efficient than dimer on a weight basis (6x on a molar basis) in displacing the monoclonal anti-Id (λ) from binding, since the monomer caused 50% inhibition at 1.6 ng/mL, whereas 18.5 ng/mL of dimer was required (Table 1). There is also a deviation from parallelism for the dimer (Fig 3), suggesting that the antigenic determinant is not identical to that of the monomer.

Normal pooled λ chains. Reactivity of the anti-Id(λ) with normal λ chains was also quantitated by the inhibition ELISA (Fig 3). These λ chains were obtained from normal IgGλ by reduction and alkylation of the disulfide bonds followed by separation in 1 mol/L propionic acid and are therefore monomeric. It was found that 3.3 x 10⁶ ng/mL...
was required for 50% inhibition (Table 1), i.e., only one molecule in about 20,000 carries the same idiotypic determinant as the λd.

**Tumor-derived IgMId.** The rescued IgMId was found to be predominantly pentameric from its elution curve on AcA 22; its ability to displace the anti-Id(λ) from its binding to λId antigen is shown in Fig 3. The amount of IgMId is given in terms of the content of the λ chain for direct comparison, and it can be seen (Table 1) that 2.5 ng/mL is required for 50% inhibition, a value close to monomer and much more efficient that dimeric λId.

It was also found that normal serum (1 in 10) failed to inhibit binding, whereas LP’s serum inhibited binding by 50% at 1 in 640 dilution.

**Additive binding to IgMId by anti-Id (λ) and anti-Id (μ + λ).** This was investigated by ELISA using limited antigen on the plate (25 ng/mL of IgMId coated indirectly) and finding a saturating concentration of anti-Id (μ + λ) (2.7 μg/mL). The anti-Id (λ) was then added to this in increasing amounts to see if additional binding could occur; the results are shown in Fig 4. Clearly the binding of anti-Id (λ) is independent of the presence of anti-Id (μ + λ), since the binding curve is very close to the calculated additive curve. The graph also illustrates that the avidity of the anti-Id(λ) is considerably less than that of the highly avid anti-Id (μ + λ), since the latter reached saturation of bound IgMId at 2.7 μg/mL, whereas the former was still not saturating at 100 μg/mL. However, the amount required for binding to cells (Fig 1) was 24 μg/mL, well within a useable range. There was no significant binding of an irrelevant mouse MoAb at these concentrations.

**DISCUSSION**

A monoclonal anti-idiotypic antibody has been raised against tumor-derived λ light chain from a patient with CLL. The antibody is specific for idiotypic determinants on the λ chain but also recognizes λ chain in combination with μ chain either as soluble idiotypic IgM or on the surface of tumor cells. These properties are similar to those of polyclonal anti-idiotypes previously raised against tumor-derived light chains from four patients with CLL and demonstrate that this dual recognition is due to a single antibody. The study also confirms the generality of the approach and, since clones showing dual specificity were not rare, suggests the possibility of using such antibodies clinically.

However, there are two possible problems. First, more aggressive immunization is required for light chain as compared to IgM. This could perhaps be solved by coupling light chain to a carrier such as keyhole limpet haemocyanin. The second problem is that the antibody studied had a considerably lower avidity for idiotypic IgM than did the antibody against heavy plus light chain (combined) idiotype, the latter having a very high avidity. Since antibody avidities are variable, this need not be a general property, but more antibodies need to be studied.

Using this MoAb it has been possible to investigate further the nature of the antigen recognized. In the more common situation where antibodies are raised against combined idiotype, they rarely bind to free light chain, more often recognizing determinants dependent on a conformation involving heavy and light chains. This has also been found for anti-idiotypes raised against IgM idiotype in this laboratory, including the one described in this report. Thus the idiotypic determinants present on the isolated light chains that can induce antibodies that recognize IgM, and therefore must be displayed on the IgM molecule, are either pre-empted by other immunogenic structures or are not detected in the assay systems used. One exception to the failure of anti-idiotype raised against IgM to recognize free light chain is seen for rheumatoid factors (RFs). The existence of a major cross-reacting idiotype (Wa) among human monoclonal IgM anti-IgG autoantibodies from unrelated individuals with cryoglobulinemia was first described using polyclonal antisera. More recently a MoAb generated against this IgM idiotype was shown to react with isolated κ chains from those IgM-RFs expressing the idiotype. The relevant deter-
MONOCLONAL ANTI–LIGHT CHAIN IDIOTYPE

923

minant was localized to the second complementarity-determining region of the κ chain and antibody raised against the isolated peptide bound to the IgM-RFs.

The use of the inhibition ELISA has allowed quantitation of the degree of binding of the antibody to various λ-containing molecules. The first point is that since it only recognized 1 in 20,000 molecules of normal λ, it can be considered to be idiotype specific. Second, binding by monomeric λId, which in antigen excess could be twice as efficient as dimer on a weight basis if the determinant is equally exposed on the two molecular species, is 12 times more efficient, indicating that masking or distortion of the determinant occurs during dimerization. However, the λ component of IgMId is almost as efficient as monomeric λ, suggesting that the determinant is exposed while λId is combined with μ chain and that, unlike the Mcg light chain studied by x-ray diffraction,20 the dimer does not assume a similar conformation to the μ-κ combination. The generality of this is unknown but raises the possibility that one of the reasons for the failure of polyclonal anti-IgM idiotypes to recognize free light chain could be due to the use of dimeric light chains in the test system.

Thus the earlier findings, reported in all four cases of CLL investigated9 and in an animal lymphoma,21 that polyclonal antibodies raised against idiotypic light chains will recognize idiotypic IgM have been confirmed using MoAb and should open the possibility of using such reagents for monitoring and perhaps treating B cell lymphoma.

ACKNOWLEDGMENT

We are grateful to H. McBride for raising the monoclonal anti-IgM idiotype, to Dr D.B. Jones for carrying out the tissue section staining, to A. Worth for help with the FACS analysis, and to M. Power for some protein preparations.

REFERENCES

21. Stevenson FK, Elliott EV, Stevenson GT: Some effects on leukaemic B lymphocytes of antibodies to defined regions of their surface immunoglobulin. Immunology 32:549, 1977

From www.bloodjournal.org by guest on October 21, 2017. For personal use only.
Monoclonal anti-light chain idioype as a tumor-specific probe for human neoplastic B lymphocytes

M Wrightham, AL Tutt, MJ Glennie, TJ Hamblin, GT Stevenson and FK Stevenson