Monoclonal Anti-Light Chain Idiotypic 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 idiotypic 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 × 10^5 molecules of pooled normal λ chains, thus establishing its specificity for a private idiotypic 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 idiotypic 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 idiotypic 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-idiotypic antibodies that usually recognize the heavy and light chain combination.

MATERIALS AND METHODS

Patient and Cell Preparation

The male patient LP, aged 50 years, presented with typical CLL with a WBC of 50 × 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 IgMDX. 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 (×100) urine was analyzed by isoelectric focusing (IEF) with immunofixation as described. Preparation of the idiotypic 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-κ to remove κ 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-1 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 antiserum IgG (5 mL). The purity of the final product was examined by IEF, and amounts were noted.

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measured at each stage by enzyme-linked immunosorbent assay (ELISA). Separation of disulfide-bonded dimeric from monomeric λ 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°C. To obtain normal λ chains, IgGλ was first prepared from normal human IgG by immunosorption. This IgGλ was then reduced and alkylated and the monomeric λ chains separated in propionic acid as above.

Monoclonal Anti-idiotypes

Anti-λ chain idiotype (anti-Id λ.) The isolated urinary λ chain was used to immunize a BALB/c mouse with a primary subcutaneous (SC) injection of 100 μg in Freund’s complete adjuvant (CFA), followed by similar booster injections after 5, 7, and 9 weeks. The final injection was of 100 μg 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).11 The procedures for growing colonies and cloning were as described,12 selection being based on reactivity of supernatants in the ELISA system with idioptic λ chain (λId), IgMld, and irrelevant light chains. To produce larger amounts, 105 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. 

Anticombined idiotype (anti-Id [μ + λ]). To obtain IgMld 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 κ chain. Colonies producing IgMλ were cloned by limiting dilution and bound mouse Ig detected with horseradish peroxidase (HRP)-labeled rabbit antimouse Ig. (A) 1: Anti-human κ chain (10 μg/mL); 2: anti-human λ chain constant region (10 μg/mL); 3: antibody against heavy plus light chain (10 μg/mL); 4: anti-Id (λ) plus normal human serum (50%).

Production of λ Id by Tumor Cells

Neoplastic B cells from LP secreted in vitro free λ light chain (20 ng/h) and IgM (7.2 ng/h), values in the range of those found previously for patients with lymphoma. Cryostat sections (5 μm) were air-dried and stored at −70°C until required. Immediately before staining the sections were fixed in acetone for ten minutes and then stained by a two-layer immunoperoxidase technique,14 using HRP-rabbit antimouse Ig as the second layer and developing reaction product with diaminobenzidine tetrahydrochloride.

RESULTS

Production of λ Id by Tumor Cells

Neoplastic B cells from LP secreted in vitro free λ light chain (20 ng/h) and IgM (7.2 ng/h), values in the range of those found previously for patients with CLL.15 The levels of light chain (κ and λ) in a typical 24-hour urine sample from LP were 0.3 (κ) and 6.8 (λ) mg per 24 hours, which is below the detection level of most routine methods and requires sensitive IEF and immunofixation or other special techniques16 for identification. Identification of the monoclonal λ chain for LP by this technique has been reported.8 After purification of the urinary λId and passage through G-100 in 1 mol/L propionic acid, the disulfide-bonded dimer accounted for about 20% of total λ.

Monoclonal Anti-Id (λ)

In the first screen of selected hybridoma supernatants, seven clones were found to recognize λId and not pooled λ.

Fig 1. Binding of MoAbs to target lymphoma cells from patient LP. Tumor cells were exposed to mouse MoAbs, and bound mouse IgG was detected with fluorescent rabbit antimouse Ig. (A) 1: Anti-human κ chain (10 μg/mL); 2: anti-human λ chain constant region (10 μg/mL); 3: antibody against heavy plus light chain idiotype (anti-Id [μ + λ]) (10 μg/mL). (B) 1: Anti-human κ chain (10 μg/mL); 2: anti-human λ chain constant region (10 μg/mL); 3: antibody against λ chain idiotype (anti-Id [λ]) (24 μg/mL); 4: anti-Id (λ) 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 IgMA) or 23 sera from macroglobulinemic patients (10 IgMK and 13 IgMA). The subclass of the antibody was IgGi.

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 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 (A) from binding, since the monomer caused 50% inhibition at 1.6 ng/mL, whereas 18.5 ng/mL of dimer was required (Table I). There is also a deviation from parallelism for the dimer (Fig 3), suggesting that the antigenic determinant is not identical to that of λId dimer (Fig 2), suggesting that they are dimerized by disulfide bonds as has been found for most human λ chains.13

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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 10^6 ng/mL

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was required for 50% inhibition (Table 1), i.e., only one molecule in about 20,000 carries the same idiotypic determinant as the λld.

**Tumor-derived IgMld.** The rescued IgMld 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 λld antigen is shown in Fig 3. The amount of IgMld is given in terms of the content of 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 λld.

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 IgMld by anti-Id (λ) and anti-Id (μ + λ).** This was investigated by ELISA using limited antigen on the plate (25 ng/mL of IgMld 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 IgMld 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 usable 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-idiotypic binding is that 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

The use of the inhibition ELISA has allowed quantitation of the degree of binding of the antibody to various \( \lambda \)-containing molecules. The first point is that since it only recognized 1 in 20,000 molecules of normal \( \lambda \), it can be considered to be idiotype specific. Second, binding by monoclonal \( \lambda \)ld, 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 \( \lambda \) component of IgMld is almost as efficient as monomeric \( \lambda \), suggesting that the determinant is exposed while \( \lambda \)d is combined with \( \mu \) chain and that, unlike the Mcg light chain studied by x-ray diffraction, the dimer does not assume a similar conformation to the \( \mu \)-\( \lambda \) 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 investigated and in an animal lymphoma, 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.

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