Epitope Mapping and Use of Anti-Idiotypic Antibodies to the L6 Monoclonal Anticarcinoma Antibody

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ABSTRACT

Mouse monoclonal anti-idiotypic antibodies (anti-ids) were raised against L6, a murine IgG2a monoclonal antibody specific for a cell surface antigen expressed by many human carcinomas. Ten distinct anti-ids were generated. Eight anti-ids were shown to inhibit the binding of L6 to its target antigen and were characterized in detail. The heavy and light chain variable region gene segments of the monoclonal antibody L6 linked to human constant regions (chimeric L6) were expressed separately or together, to map the epitopes recognized by the anti-ids. Individual anti-ids were shown to recognize heavy chain, light chain, or combinatorial variable region determinants. Defining these specificities enabled us to select particular anti-ids for assays to monitor the pharmacokinetics of either murine or chimeric L6 antibodies in the circulation of human patients. A quantitative enzyme-linked immunosorbent assay developed with two anti-ids accurately detects less than 5 ng/ml. Anti-ids specific for light chain variable region-encoded determinants were capable of recognizing L6 antigen-binding fragments bound to the surface of human carcinoma cells. These anti-ids can be used to study the binding of chimeric L6 antibody at the surface of tumor cells in histological sections of tumor biopsies.

INTRODUCTION

The advent of mAb technology (1) created the opportunity to use antibodies as pharmaceutical reagents for the treatment of cancer and infectious diseases, as well as the modulation of the immune response in transplantation therapy and autoimmune disorders. mAbs are most commonly obtained through immunization of mice or rats. More recently, molecular genetics have been applied to generate chimeric antibody molecules (murine V-region, human C-region) which are expected to be less immunogenic and potentially more effective in humans (2–7). Additional therapeutic approaches involve the use of antibodies (or portions of antibodies) to deliver toxins or drugs (8, 9), radionuclides (10), enzymes (11, 12), or other biologically active molecules to the tumor site. Study of the tissue distribution and pharmacokinetics of clinically administered antibodies requires the development of methods to discriminate between the administered material and endogenous proteins.

mAb L6 defines an antigen which is expressed in large amounts at the surface of cells from many different human carcinomas, including breast, lung, colon, and ovary, and in only trace amounts on normal tissues (13, 14). In clinical trials the antibody has been shown to localize to tumor cells in biopsies of metastatic lesions (15). Chimeric L6 antibody has been made via recombinant DNA technology (5) and introduced into the clinic. We report the generation and characterization of monoclonal anti-ids that recognize unique antigenic determinants present on the V-regions of L6 and we demonstrate their usefulness in following the presence of either murine or chimeric L6 in the circulation of patients and on the surface of tumor cells in metastatic lesions.

MATERIALS AND METHODS

Animals, Cell Lines, and Antibodies. Six- to 8-week-old female BALB/c and C3H/HeN mice were purchased from the Animal Facilities of the Fred Hutchinson Cancer Research Center. Human colon carcinoma line H-3347 was employed for in vitro binding assays, since it expresses high levels of the antigen defined by mAb L6 (13). The human T-cell line CEM, which does not bind L6 (13), was included as a negative control. mAb L6 is a murine IgG2a which binds to a cell surface antigen expressed by most carcinomas (13). L20 is a murine IgG1 mAb which recognizes an antigen distinct from L6 antigen present on H-3347 cells and other carcinomas (13). Anti-melanoma mAb 96.5 (16) binds neither H-3347 nor CEM cells and is a murine IgG2a used as an isotype-matched control for L6 studies. F(ab) fragments were made from L6 and 96.5 by papain digestion (17) and are referred to as L6 F(ab) and 96.5 F(ab), respectively.

Generation of Anti-Idiotypic Antibodies. Our protocol for generating murine monoclonal anti-ids has been published in detail (17). BALB/c mice were immunized with 100 µg of L6 coupled to keyhole limpet hemocyanin. The first immunization was given s.c. in complete H37Ra adjuvant (Difco, Detroit, MI) and a second dose was given i.p. in incomplete Freund's adjuvant 4 weeks later. Two to four subsequent immunizations were administered i.p. with L6 in phosphate-buffered saline at 2-week intervals.

Spleens were removed 3 days after the last boost and the spleen cells were fused with NS-1 myeloma cells (ATCC TIB 18) by centrifugation with polyethylene glycol. Ten days later, hybridomas were screened by ELISA against L6 F(ab) coated onto the wells of 96-well Immulon II plates (Dynatech, Chantilly, VA). Binding was detected with either rabbit anti-mouse IgG1 or IgG3 coupled to HRP or Protein A-HRP (Zymed, South San Francisco, CA). Supernatants binding to L6 F(ab) were tested for binding to 96.5 F(ab) to exclude those which recognize constant region determinants or V-region framework epitopes not specific to L6. Hybridomas producing antibody which bound only to L6 F(ab) were cloned twice by limiting dilution and injected into pristane-primed BALB/c mice for ascites production.

Anti-ids were purified from ascites or culture supernatants by Protein A affinity chromatography and biotinylated or conjugated to FITC (18).

Assay for Ability of Anti-id to Block L6 Binding to Tumor Cells. To determine which L6 anti-ids recognize epitopes located near the antigen binding site, an inhibition assay was employed. Culture supernatants of anti-id-producing hybridomas or purified anti-ids were mixed with 1.0 µg/ml L6 and incubated for 30 min at room temperature. The mixture was then added to glutaraldehyde-fixed H-3347 cells attached to the wells of 96-well microtiter plates. Binding of L6 to tumor cells was detected by an ELISA using a goat anti-murine IgG-HRP (Zymed) as the second step. Data are expressed as the ELISA absorbance values

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2To whom requests for reprints should be addressed, at ONCOGEN, 3005 First Avenue, Seattle, WA 98121.

3The abbreviations used are: mAb, monoclonal antibody; anti-id, anti-idiotypic antibodies; Vh, heavy chain variable region; Vl, light chain variable region; Hh, heavy chain of antibody; Lh, light chain of antibody; Vh-region, variable region of antibody; F(ab), antigen-binding fragment of antibody; CDR, complementarity-determining region of antibody; HRP, horseradish peroxidase; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; ELISA, enzyme-linked immunosorbent assay; ATCC, American Type Culture Collection.

generated by L6 binding to cells in the presence and absence of inhibitiing anti-ids.

Production of Chimeric L6 and Cell Lines Producing Only One L6 V-Region. Cloning of the L6 heavy chain and light chain gene segments (VH and VL) and construction of vectors expressing human IgG1 and κ chimeric proteins will be published elsewhere. The chimeric light chain and heavy chain gene constructs were transfected into the non-immuno-globulin-producing mouse myeloma cell line P3X63Ag 8.653 (ATCC CRL 1580) using a Bio-Rad (Richmond, CA) electroporator as per manufacturer’s recommendations, with subsequent selection in mycophenolic acid. Similar transfections were performed introducing the chimeric light chain gene construct by itself into the SP2/0 mouse myeloma line (ATCC CRL 1581). The resulting cell line secretes chimeric L-chain dimers. The chimeric heavy chain construct was transfected into the J558L murine myeloma which expresses a murine λ-light chain but no heavy chain (19). The resulting cell line secretes chimeric H-chains assembled to the λ-light chain. Chimeric L6 antibody was purified on Protein A-Sepharose and biotinylated (18).

Mapping L6 Anti-ids to H-Chain, L-Chain, or Combinatorial V-Region Epitopes. Competition assays to map anti-id epitopes were performed by coating Immulon II plates with 100 µl of each anti-id at 15 µg/ml in 0.05 M Na2HCO3, pH 9.6. Wells were blocked with 300 µl of specimen diluent (Genetic Systems, Seattle, WA) and washed with 0.5% Tween 20 in phosphate-buffered saline. One hundred µl of unla- beled competitor were added at concentrations ranging between 5 ng/ ml and 5 µg/ml. The unlabeled competitors were either culture supernatants from transfectants expressing the L6 heavy chain antibody associated with the J558L light chain or the L6 chimeric light chain dimer or culture medium spiked with purified chimeric L6. After 30 min at 37°C, 50 µg of a 50 ng/ml solution of purified biotinylated chimeric L6 were added to the 100-µl volume already present in the well. This mixture was incubated for 1 h at 37°C, after which the plate was washed. Avidin-HRP (TAGO, Burlingame, CA) diluted 1:1000 was added and incubated for 30 min at room temperature. The plates were washed again and developed with 3,3′,5,5′-tetramethylbenzidine chromogen in buffered substrate (Genetic Systems), after which the reaction was stopped with 100 µl of 3 N H2SO4. Plates were read on a Titertek microplate reader (Winooski, VT).

Anti-id ELISA to Quantify Murine and Chimeric L6. Anti-ids against two different determinant, one on the H-chain (antibody 13) and one on the L-chain (antibody 1), were chosen to develop a quantitative sandwich immunoassay for L6 and chimeric L6. Wells of a microtiter plate were coated with 100 µl of anti-id 1 at 2 µg/ml in bicarbonate buffer, blocked, and washed as above. A standard curve was constructed from the purified antibody to be measured. Samples to be quantified were diluted so that the measured values fell within the linear range of the standard curve. After serum and standard curve samples were incubated in the plate (overnight at 4°C), plates were washed and 100 µl of 100 ng/ml biotinylated antibody 13 in conjugate diluent (Genetic Systems) were added and incubated for 1 h at room temperature. After another wash, Vectastain (Vector Laboratories, Burlingame, CA) was added and incubated for 30 min at room temperature. The plates were washed again, developed with 3,3′,5,5′-tetramethylbenzidine chromogen in buffered substrate, stopped with H2SO4, and read as described above.

Anti-IgG2a ELISA to Quantify L6. This assay was performed as above except that the plate-coating antibodies were affinity-purified goat anti-murine IgG2a (FischerBiotek, Orangeburg, NY) at 1 µg/ml and the second step was the HRP conjugate of the same antibodies used at a 1:3000 dilution.

Determination of Which Anti-Ids Bind to L6 Previously Bound to Cellular Antigen. H-3347 cells (5 x 10^5/ml) were saturated with L6 (30 µg/ml). After 30 min at 4°C, the cells were washed to remove any unbound F(ab). Purified anti-ids labeled with fluorescein isothio-cyanate were added in increasing concentrations and incubated at 4°C for 30 min. Cells were washed and the binding of anti-id to the cell-bound L6 was detected using an EPICS-C FACs. The mean fluorescence intensity of the FITC-anti-id signal was converted to linear fluorescence equivalents according to a predetermined calibration curve, to illustrate the amount of anti-id bound as a function of concentration.

Immunohistology. A modification of the peroxidase-antiperoxidase technique of Sternberger (20) was used, as previously described (13, 21). Frozen sections were prepared from carcinoindomas and normal tissues and exposed to either mouse L6, chimeric L6, or L20. This was followed either by a HRP-conjugated goat antiserum to mouse immunoglobulin or by biotinylated anti-ids followed by Vectastain (Vector Laboratories), after which chromogenic reagents were added as described previously (13). Coded slides were read under the microscope in a “blind” fashion, and the staining of L6-positive cells was scored as in the past (13).

RESULTS

Generation and Initial Characterization of Anti-Idiotypes. Synthetic mice (BALB/c) were immunized with L6 to elicit anti-idiotypic antibodies. In fusion experiments with eight mice, 24 hybridomas were originally identified that produced mAbs which bound L6 F(ab) but not F(ab) from a different, isotype-matched, control antibody, 96.5. Some of these were indistinguishable upon further characterization and data are presented for only a single member of a group of similar antibodies, if they came from the same mouse. By these criteria, 10 distinct mAbs were identified.

To determine which anti-ids bind at or near the antigen binding site of L6, we tested each anti-id for its ability to block binding of L6 to cells. Culture supernatants from 8 of the 10 anti-ids tested were able to inhibit. These eight antibodies were then purified by Protein A affinity chromatography and compared quantitatively (Fig. 1). A 5- or 50-fold excess of each anti-id was preincubated with 1 µg/ml L6, an amount that does not saturate all antigen sites on H-3347 cells. The data confirmed that these anti-ids are capable of inhibiting the binding of L6 to tumor cells, suggesting that they recognize determinants located near the antigen-combining site. The quantitative comparison also revealed differences among the anti-ids in their ability to inhibit L6 binding. In particular, antibodies 7 and 9 appeared relatively weaker than the others.

Mapping the Anti-id Epitopes to the Variable Region of L6 Heavy or Light Chain or to a Combinatorial Determinant. The cloned variable region gene segments linked to human constant regions were expressed together or separately, as described in “Materials and Methods,” and used to further characterize the location of determinants recognized by the L6 anti-ids. Chimeric mAb L6, chimeric L6 light chain dimer, and chimeric L6 heavy chain associated with an irrelevant light chain (J558L murine λ-light chain) were used to compete with biotinylated chimeric L6 for binding to each anti-id. Fig. 2 gives examples of each of the three recognition patterns observed, representing

![Graph showing anti-idotype distribution](image-url)
OD 0.3-

chimeric L6 light chain dimer (•) were added. After preincubation, biotinylated
dimer (*). chimeric L6 heavy chain associated with J558 L-chain (A), or
epitopes requiring co-expression of both chains. Each anti-id was coated to the
chimeric L6 (SO ng/ml) was added and its binding was detected as described in"Materials and Methods." The data are presented as the mean absorbance of
wells of microtiter plates and unlabeled inhibiting proteins consisting of either
chain, or to a determinant requiring the association of bothvariable regions (a "combinatorial" determinant). All of the
of inhibitor concentration.

This binding was inhibited by unlabeled chimeric L6 but not by
anti-ids could bind to the biotinylated chimeric L6 molecule
epitopes which map to the V-region of the H-chain, to the L-
chain, or to a determinant requiring the association of both variable regions (a “combinatorial” determinant). All of the
anti-ids could bind to the biotinylated chimeric L6 molecule and thus recognize the product of the cloned L6 V-regions. This binding was inhibited by unlabeled chimeric L6 but not by
an irrelevant human IgG1 (data not shown). The binding of
four of the anti-ids (antibodies 1, 7, 9, and 15) was inhibited by free chimeric L6 L-chain but not by chimeric L6 H-chain associated with murine λ-1 light chain. These four anti-ids thus recognize determinants associated with the L6 L-chain V-region.
One of the remaining anti-ids (antibody 13) bound to the
L6 H-chain V-region associated with an irrelevant light chain, whereas the other three (antibodies 3, 1, and 14) recognized

Table 1 Summary of data on 10 anti-ids specific for L6

<table>
<thead>
<tr>
<th>Anti-id</th>
<th>Isotype</th>
<th>Inhibits L6 binding to antigen</th>
<th>Binds to L6 saturated cells</th>
<th>Variable region specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG2b</td>
<td>Yes</td>
<td>Yes</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>IgG2a</td>
<td>Yes</td>
<td>No</td>
<td>H &amp; L</td>
</tr>
<tr>
<td>5</td>
<td>IgG3</td>
<td>No</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>IgG1</td>
<td>Yes</td>
<td>Yes</td>
<td>L</td>
</tr>
<tr>
<td>9</td>
<td>IgG1</td>
<td>Yes</td>
<td>Yes</td>
<td>L</td>
</tr>
<tr>
<td>11</td>
<td>IgG1</td>
<td>Yes</td>
<td>No</td>
<td>H &amp; L</td>
</tr>
<tr>
<td>13</td>
<td>IgG1</td>
<td>Yes</td>
<td>No</td>
<td>H</td>
</tr>
<tr>
<td>14</td>
<td>IgG1</td>
<td>Yes</td>
<td>No</td>
<td>H &amp; L</td>
</tr>
<tr>
<td>15</td>
<td>IgG2a</td>
<td>Yes</td>
<td>Yes</td>
<td>L</td>
</tr>
<tr>
<td>16</td>
<td>IgM</td>
<td>No</td>
<td>ND</td>
<td>ND</td>
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</table>

ND, not determined.

**Fig. 3. Standard curve for the anti-id ELISA to quantify chimeric L6. The**

Quantitation of L6 and Chimeric L6 in Serum. Two anti-ids
recognizing distinct epitopes (one to VH and one to VL) were
chosen to develop a quantitative sandwich immunoassay for the
detection of murine chimeric L6 antibody. The mapping of
the epitopes enabled us to choose two monoclonal antibodies
likely to bind to epitopes distant enough from each other that
the anti-ids would not sterically block each other's binding. By
making our choice based on the mapping, we eliminated the
need either to test the 90 possible pairwise combinations of 10
anti-ids or to do extensive competition experiments between
them. Furthermore, by choosing monoclonal antibodies against
separate determinants on the L6 H-chain and L-chain, we felt
we optimized the chances that the assay would be highly specific
for the L6 immunoglobulin and not recognize antibodies that
use related H- or L-chain V-regions.

**Fig. 3 shows the standard curve for this assay using known quantities of purified chimeric L6, demonstrating that the linear range extends between 2.5 and 50 ng/ml. Since a patient sample of human serum requires a 2-fold dilution to eliminate nonspecific interferences, the assay has a lower limit of detection of 5 ng/ml in serum. We measured the accuracy of the assay at both relatively high and low concentrations of L6, since the concentration of mAb in patients varies widely depending on the dose and time after infusion. Samples of normal serum from different individuals were prepared containing either 100 ng/ml or 50 µg/ml chimeric L6 and then were quantified. In 20 replicates of each sample, the assay detected 96 ng/ml ±11.4% and 50

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μg/ml ± 10.8% chimeric L6, respectively. Thus, the assay is both accurate and precise over a 500-fold range.

Assays employing anti-ids which bind at or near the antigen binding site of an antitumor mAb could be misleading, if antigen were shed into the serum or if the anti-ids cross-react with human serum proteins. The anti-idiotype assay was, therefore, compared with a similar assay for murine IgG2a that employs conventional polyclonal reagents. Ten serum samples were selected from six different patients with carcinomas expressing the L6 antigen who had received infusions of murine L6. The L6 concentration was quantified using both assays.

Fig. 4 shows the correlation between the levels of L6 measured in the two assays over the range of concentrations that occurred in these samples, roughly 100 ng/ml to 500 μg/ml. The slope of the line determined by linear regression is 0.98, in agreement with the expected value of 1.00. The correlation coefficient (r²) between the two sets of values calculated by the method of least squares is 0.997. Thus, the anti-idiotype assay is as accurate for the determination of serum concentrations of therapeutically administered antibody as an assay using more conventional polyclonal reagents.

Identification of Anti-ids Capable of Binding to L6 F(ab) Preceding Bound to Tumor Antigen. To determine whether the anti-ids that block antigen recognition by L6 would be inhibited by antigen from binding to L6, H-3347 cells were incubated with a saturating amount of L6 F(ab) and washed. F(ab) was used to ensure that any subsequent binding by an anti-idiotype would be to a variable region already bound to cellular antigen and not to the other “arm” of a whole antibody. Increasing concentrations of FITC-labeled anti-idiotype were then added to the cells and the amount bound to L6 F(ab) was quantified by FACS analysis (Fig. 5). Four of the anti-ids that block L6 binding to tumor cells do bind to L6 F(ab) attached to the cell surface and four do not. As summarized in Table 1, the four antibodies able to bind to antigen-bound L6 F(ab) are the same four that recognize V_L-encoded epitopes. Neither V_H-nor combinatorial-specific anti-ids possess this activity. This may suggest that the epitopes recognized by these antibodies are more closely associated with the antigen binding site of L6.

It is interesting to note that two of the anti-ids that bind to L6 on cells, antibodies 15 and 1, saturate the cells at much lower concentrations than do antibodies 7 and 9. This is consistent with the earlier observation that antibodies 7 and 9 were relatively weaker at blocking the binding of L6 to cells than the other anti-ids.

Fig. 4. Correlation between L6 concentrations measured in clinical samples by the anti-idiotype ELISA and an assay for murine IgG2a. Ten serum samples from six patients infused with murine L6 were quantified by both assays. The L6 concentration measured in each sample by the anti-idiotype assay is plotted as a function of that measured in the IgG2a assay.

Fig. 5. Ability of anti-ids to bind to L6 F(ab) attached to tumor antigen. Tumor cells were coated with nonsaturating levels of L6 F(ab) and washed. FITC-labeled anti-ids were then added to the cells and the ability of the anti-ids to recognize antigen-bound L6 was quantified in a FACS. Data are presented as the mean fluorescence intensity of the peak representing 10⁶ to 10⁵ cells converted to linear fluorescence equivalents (arbitrary units). The amount of anti-idiotype is shown as a function of anti-idiotype concentration.

Anti-ids Can Be Used to Detect the Presence of L6 and Chimeric L6 on the Surface of Tumor Cells in Biopsies. Since some of the anti-ids bound to L6 F(ab) attached to the surface of tumor cells, it became possible to develop immunohistological assays to detect the binding of murine or chimeric L6 to tumor cells following its i.v. infusion into cancer patients. There is a particular need for such assays when working with chimeric mAbs, since the anti-mouse immunoglobulin reagents frequently used to detect the binding of murine mAbs to tumor cells cannot be applied. Anti-human immunoglobulin reagents directed to the constant region cannot distinguish between the administered protein and the patient's own antibody in biopsy specimens.

Anti-ids 1 and 15 were both found to be useful for demonstrating the tumor-specific binding of both murine and chimeric L6 in sections from antigen-positive carcinoma cells. Fig. 6 illustrates the use of anti-idiotype 15 to detect the binding of chimeric L6 applied to tumor sections. Shown are two serial sections of a biopsy taken from a metastatic lesion of an L6-positive carcinoma of the breast. The sections were treated with either chimeric L6 (Fig. 6A) or a control mAb, L20, that recognizes a tumor antigen present on this tumor different from L6 antigen (Fig. 6B). Sections were then treated with biotinylated anti-idiotype followed by avidin-HRP and a chromogenic substrate. The anti-idiotype specifically demonstrates the presence of chimeric L6 on the surface of tumor cells. The normal stroma is not stained, nor is the tumor-binding antibody of a different specificity. Additional experiments using murine L6 showed that tumor cells expressing the L6 antigen were stained to the same extent with the anti-idiotype reagents as with HRP-labeled goat antibodies to mouse immunoglobulin. Anti-idiotype 13, which maps to the H-chain and recognizes free but not cell surface-bound L6, could not be used for immunohistochemical staining (data not shown).

DISCUSSION

mAbs are being developed as therapeutic reagents for a variety of human diseases and particularly for cancer therapy (22). Understanding the clearance, biodistribution, and localization to tumor cells of therapeutic antibodies is of critical importance for developing effective clinical protocols. Genetic engineering has provided the means to generate antibody molecules, such as chimeric antibodies, that more closely resemble human proteins, in the hope of improving effector functions and minimizing immunological reactions. Reagents that can
distinguish between the administered antibody and endogenous immunoglobulin are crucial but are more difficult to come by as the therapeutic antibodies more closely resemble normal human serum immunoglobulin. Reagents recognizing unique idiotypic determinants on the therapeutic antibody are ideal for overcoming this problem. LoBuglio et al. (23) have used rabbit anti-idiotypic antiserum to study the pharmacokinetics of the 17-1A chimeric antibody against gastrointestinal tumor-associated antigens.

We have generated 24 monoclonal anti-ids which bind to the V-regions of L6, a murine mAb recognizing a human tumor-associated antigen. Ten of the anti-ids were studied further, after elimination of likely duplicates of the same anti-id clones. Eight of these 10 anti-ids inhibited the binding of L6 to its target antigen and so probably bind at or near the CDRs of L6. This large percentage of anti-ids against the antigen binding site may result from our choice to immunize syngeneic mice to obtain anti-ids rather than other strains of mice or rats. In syngeneic mice the hypervariable regions probably represent the most unique and possibly the most immunogenic part of the molecule.

The two anti-ids that do not inhibit binding of L6 to antigen may be specific for L6 V-region framework determinants but, because such epitopes seem more likely to be shared with other immunoglobulins and conceivably with human framework regions, we chose to focus on the anti-ids mapping near the L6 CDR. Examination of the database of human and murine V-region sequences reveals that many of the members of the human and murine V-region subgroups share 80% or more sequence homology in the framework residues (24).

We have shown which anti-idiotypic antibodies bind to H-chain, L-chain, or combinatorial determinants. Although this approach requires cloning the murine H- and L-chain V-region genes, these will be available for many therapeutic antibodies, where producing chimeras is desirable. Defining the position of epitopes recognized by the eight antigen-blocking anti-ids revealed three recognition patterns. Four of the anti-ids were shown to bind to VL-encoded determinants, and one bound to a VH-encoded determinant. Three anti-ids bound only to a combination of the L6 variable regions.

This information has proved valuable for choosing which anti-ids to use for constructing immunoassays for monitoring the pharmacokinetics and tumor binding of L6 and chimeric L6 given to patients. An ELISA specific for two determinants closely related to the antigen binding site of L6 was formulated, which can be used to quantify either murine or chimeric L6 in the circulation of patients given infusions of the respective mAb. It may be that these or similar anti-idiotypic antibodies would also be able to recognize the L6 CDRs even when transplanted onto human framework regions in a "humanized" version of L6 (25). L6-related anti-ids can also be useful for recognizing the product of various recombinant DNA constructs that combine the L6 variable regions with other proteins.

The VL-specific anti-ids did not compete reciprocally with antigen for binding to L6. That is, they were capable of blocking L6 from binding to antigen but could still recognize their epitopes after the L6-combining site became occupied by antigen. Although the mechanism for this effect is unclear, similar observations have been made by others when mapping a battery of mAbs to a given antigen (26). The effect could result from differences in affinities between the binding pairs, steric hindrance, or perhaps allosteric changes that occur with binding.

Whatever the mechanism, this property is useful, because it allows the development of an assay to detect mAb L6 when bound to tumor cells. Furthermore, the fact that all four VL-specific anti-ids are capable of binding cell surface F(ab) suggests that the H-chain V-region of L6 may be more important for antigen binding.

The availability of reagents to monitor the presence of chimeric antibody on the surface of tumor cells taken from patient biopsies offers a unique opportunity to study the pharmacology of this chimeric antibody during clinical trials. The ability to directly assess the amount of specific localization to tumor and the cell surface half-life of the chimeric antibody, combined with an accurate quantitation of the level of antibody present in patient circulation, will provide a more detailed analysis of the distribution and mechanisms of action of clinically relevant antibodies.

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REFERENCES


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