Human Monoclonal Antibody Identified an Immunoreactive Tetrapeptide Sequence (Lys-Tyr-Gln-Ile) in Mr 43,000 Protein of Human Melanoma

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ABSTRACT

The human monoclonal antibody (HuMAb) L92 reacts to an Mr 43,000 protein associated with human melanoma. To identify the gene encoding its antigenic epitope, a complementary DNA expression library constructed from the human melanoma cell line UCLASO M14 was screened with HuMAb L92. DNA sequence analysis of the isolated clone revealed that the immunoreactive peptide was composed of 10 amino acids (QDLTMKYOIF). The peptide was expressed in Escherichia coli with β-galactosidase as a fused protein. There is no homology between the cloned sequence and other reported DNA sequences. Western blot analysis showed that the fused protein had specific binding to HuMAb L92. An antigen-encoding peptide with 10 amino acids was synthesized and tested for its immunoreactivity in vitro. HuMAb L92 reacted specifically to the 10-amino acid peptide in both an antibody-binding inhibition to the Mr 43,000 protein and a solid-phase enzyme-linked immunosorbent assay. Using several truncated fusion proteins, we found the minimum number of amino acids required for the antibody binding to be 4 (KVQI). These results suggest that the identified peptide sequence encodes the antigenic epitope of the Mr 43,000 protein.

INTRODUCTION

For the last two decades, melanoma, which is one of the most immunogenic human cancers, has been the prime target for investigational immunotherapy studies. Treatment of melanoma patients with lymphokines, cytotoxic T-cells, tumor vaccine, or other biological response modifiers has resulted in complete or partial cure of disseminated melanoma with a response rate of up to 30%. Specific immunity against melanoma is believed to play a major role in tumor eradication. Nevertheless, the antigens responsible for tumor killing have not been defined in many of these trials. The criteria in past studies for the selection of patients for the clinical trials have not included antigenic expression of in vivo melanoma. For immunotherapy of human cancer to improve, the mechanisms of antitumor action, especially the nature of targeted antigens or epitopes, must be defined.

A number of melanoma-associated antigens have been identified, and yet many of them may be indifferent to tumor rejection because of nonimmunogenicity in man or T-cell independence. A preferable probe for identifying antigens associated with tumor rejection is to use human cytotoxic T-cell lines that have the ability to kill autologous tumor cell lines. Boon et al. (1, 2) used CTL clones established from melanoma patients to identify tumor-associated genes involved in the recognition and cytotoxicity of melanoma cells. This technique, which has now been tested by many investigators, requires several crucial experimental steps: the successful establishment of antigen-negative tumor cell lines that are autologous to antigen-positive tumor cell lines, the establishment of long-lasting tumor-associated CTL lines, and the transfection of thousands of DNA cosmids into the antigen-negative tumor cell lines and the screening of them for antigen expression by the CTL. Our strategy, on the other hand, has been to use human monoclonal antibodies established from B-cells of melanoma patients (3). Using this approach, we have identified two highly immunogenic melanoma-associated antigens, gangliosides Gd2 and Gd1b. Administration of these antigens to patients induces a high titer of antibody response (6, 7). It should be noted that two other melanoma-associated gangliosides, Gd3 and Gd3, which were initially identified by murine monoclonal antibodies, are either not immunogenic or are much less immunogenic in man (6, 8). These results suggest that antigens immunogenic in man are most frequently detected when human T-cells or antibodies are utilized as detecting probes.

In this report, we have identified a tumor-associated peptide antigen using a human monoclonal antibody derived from a melanoma patient. Whether or not the peptide is responsible for T-cell responses needs to be investigated; however, the protein molecule containing the epitope responsible for the antibody may also be responsible for the T-cell responses, since an antigenic protein molecule can potentially induce both humoral and T-cell responses by utilizing the same sequence peptide, the epitope with additional sequence, or different epitopes within the same molecule. The peptide identified in the present study using a human monoclonal antibody consists of only 4 amino acids. Our preliminary study demonstrated that these 4 amino acids are also essential components for recognition and lysis by T-cells.

MATERIALS AND METHODS

Human Monoclonal Antibody. Epstein-Barr virus transformation techniques (3) were used to establish a B-cell line that produces HuMAb L92 from PBL of a melanoma patient. In the immune adherent assay that detects surface antigens (3), HuMAb L92 (8 μg/ml) was shown to react to several different human cancer cell lines, including melanoma (10 of 18), renal cell carcinoma (4 of 4), gastrointestinal cancer (2 of 3), lung carcinoma (1 of 3), and neuroblastoma (1 of 2). Although the immune adherent assay revealed that no normal cells reacted with HuMAb L92, Western blot analysis of cell lysates showed a positive reaction not only to all human tumor cells but also to normal cells, including PBL. When biopsied melanoma tissue sections were tested by immunohistochemical analysis, HuMAb L92 bound to melanoma tissues but not to adjacent normal tissues. Previously, the HuMabs characterized by our laboratory were to carbohydrate epitopes (3–5). To test the possibility that HuMAb L92 may recognize carbohydrate molecules, we treated antigen-positive melanoma cells with several standard glycosidases. The ability of antigen(s) to bind with HuMAb L92 was not altered by treatment with glycosidases such as β-galactosidase, α-mannose, neuraminidase, and α-fucosidase. HuMAb L92 was devoid of reactivity to gangliosides and neutral glycolipids purified from antigen-positive melanoma cells.

Immunohistochemical Analysis. SDS-PAGE followed by Western blot analysis was carried out to detect antigen molecules reactive to HuMAb L92. Sample protein solutions in 50 mM Tris-HCl (pH 6.8; 5% β-mercaptoethanol-2% SDS-0.1% Bromophenol Blue-10% glycerol) were boiled for 5 min. Protein separation utilized a 4–20% gradient SDS-PAGE. Proteins were transferred to a nitrocellulose filter and sequentially reacted with HuMAb L92 and peroxidase-conjugated antibody (Boehringer Mannheim). Coloring reaction was

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4 The abbreviations used are: CTL, cytotoxic T-lymphocyte; HuMAb, human monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; PBL, peripheral blood lymphocytes; FCS, fetal calf serum; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; cDNA, complementary DNA; IPTG, isopropylthiogalactosidase; LB, Luria broth; GST, glutathione S-transferase; NP-40, Nonidet P-40.

carried out with 4-chloro-1-naphthol. Peptide ELISA was performed by use of Reacti-Bind Plate (Pierce), as described in the instruction manual by the manufacturer. Briefly, synthetic peptide was dissolved in 0.1 M sodium phosphate-0.15 M NaCl, pH 7.2, and added to ELISA plates (100 μl well) by overnight incubation at 4°C. ELISA was then carried out with HuMAb L92 or L612 using peroxidase-conjugated goat anti-human IgM antibody.

Indirect immunoprecipitation of in vivo labeling cells with [35S]methylene was performed to identify protein molecules reactive to HuMAb L92. Briefly, M14 melanoma cells were cultured in RPMI 1640 medium supplemented with 10% FCS in T25 culture flasks (Costar) until 70% confluence. The cells were then washed twice with a media preparation consisting of methionine-deficient RPMI 1640 medium (Sigma, St. Louis, MO) dissolved in 1 liter double-distilled water, to which glutamic acid (0.02 g/liter), lysine (0.04 g/liter), leucine (0.05 g/liter), and sodium bicarbonate (2 g/liter) were added. Two ml of this preparation, supplemented further with 10% dialyzed fetal calf serum (Sigma), were added to the flasks, which were subsequently incubated with 1.85 MBq of [35S]methylene (Amersham) for 4 h. After the cells were harvested and washed twice with phosphate-buffered saline, they were lysed with 100 μl lysis buffer consisting of 50 mM Tris-HCl (pH 8.0)-5 mM NaCl-0.2% sodium azide-0.1% SDS-100 μg/ml phenylmethylsulfonyl fluoride-1% NP-40.5% sodium deoxycholate.

Forty μl of the cell lysate were diluted to 300 μl with lysis buffer. The lysate was then precleared by incubation with 20 μl of goat anti-human IgM-biotin (Boehringer) for 1 h on ice and then with 100 μl of Streptavidin-agarose (Sigma) for an additional 1 h. The supernatant was collected via centrifugation and divided into 3 aliquots. One aliquot was incubated with 10 μg of HuMAb L92. The second aliquot was incubated with HuMAb L612 (unrelated antibody), and the third aliquot was incubated without antibody for 1 h on ice. Ten μl of anti-human IgM-biotin were added to each of the three aliquots and incubated for an additional 1 h. Ten μl of Streptavidin-agarose was then added to each aliquot and incubated for another 1 h. The antigen-anti-IgM-biotin-Streptavidin-agarose complex was pelleted via centrifugation and washed 3 times with 1 ml of lysis buffer followed by washing with 1 ml of 10 mM Tris-HCl (pH 7.0)-1% NP-40. Immunoprecipitates were analyzed by 4–20% gradient SDS-PAGE under reducing conditions. The gel was treated with Amplify (Amersham) and processed for autoradiography.

Construction of a cDNA Expression Library from Human Melanoma Cell Line M14. M14 cells were cultured in RPMI 1640 medium supplemented with 10% FCS and antibiotic (GIBCO, Grand Island, NY). Total RNA was isolated from 5 × 107 M14 cells using guanidium isothiocyanate (9). Poly(A)+ RNA was prepared using the Poly A Tract mRNA Isolation System (Promega). cDNA was prepared using the Copy Kit (Invitrogen). After the EcoRI adaptor was ligated to both ends, cDNA was inserted into the EcoRI site of Ag/c 1 phage DNA. In vitro packaging was performed using GigaPak II Gold (Stratagene). The library was amplified once on solid media before screening.

Screening of cDNA Expression Library with HuMAb L92. Escherichia coli Y1090 was cultured in an LB supplemented with 0.2% maltose and 0.5% MgSO4 until A600 reached 0.5 at 37°C. Cells were collected and suspended in half of the original volume of culture medium of 10 mM MgSO4. Cells were infected by a phage library and plated on LB agar plates (approximately 20,000 plaque-forming unit plate). The plates were incubated for 3.5 h at 42°C. After nitrocellulose filters which had been immersed in 10 mM IPTG were placed on the plaque, the plates were incubated for an additional 4 h at 37°C. The filters were subsequently immunostained by HuMAb L92 and peroxidase-conjugated goat anti-human IgM antibody. The coloring reaction was carried out with 4-chloro-1-naphthol. The plaques, which corresponded to a positive signal on the filters, were removed from the agar plates and eluted to prepare the phage stock. The screening procedure was repeated until a homogeneous population of immunopositive recombinant bacteriophages was obtained.

Sequencing of cDNA Insert. The nucleotide sequence of the cDNA insert in the recombinant Agt11 phage was determined using the Double Stranded DNA Cycle Sequencing System (BRL, Gaithersburg, MD).

Expression of Fusion Proteins. Fifty μl of recombinant phage lysogen which had been cultured overnight at 32°C were inoculated in 5 ml of LB/ampicillin liquid medium and cultured at 32°C with shaking. When the A600 reached 0.5, the temperature was shifted to 42°C for 10 min. A final concentration of 1 mM IPTG was added to the culture. Cells were cultured at 37°C for 2 h, harvested via centrifugation, and analyzed by SDS-PAGE. The synthetic oligonucleotides coding for individual epitope sequences were also cloned in the GST fusion protein expression vector pGEX-2T and transformed in the E. coli strain XL-1 blue.

RESULTS

Detection of Immunoreactive Molecule against HuMAb L92 in Melanoma Cell Lysates by Western Blot Analysis. Human melanoma cell lines M14 and M12 and human fetal brain cells were tested for their reactivity to HuMAb L92 in Western blotting. Two different medium supplements, FCS and human AB serum, were used in the case of the M14 melanoma cell line. To prepare the lysates, cells were lysed in a lysis solution containing NP-40, SDS, and deoxycholate. The blot shows a discrete band in all of the different cell lysates (Fig. 1A). In a control experiment, a purified human IgM was tested against the same cell lysates (Fig. 1B). No corresponding band was detected on the gel. The molecular weight of the reactive protein was 43,000 as estimated from its mobility in the gel. Cell lysates obtained from M14 melanoma cells grown in human serum medium had the same band, indicating that the protein had not derived from FCS in the culture medium.

M. 43,000 Protein Detected by Immunoprecipitation from [35S]Methionine-labeled M14 Cells. M14 lysates were prepared after the cells were pulsed with [35S]methionine for 4 h. After the cell lysate was precleared with anti-human IgM-biotin and Streptavidin-agarose, HuMAb L92 was added and incubated. Either the unrelated HuMAb L612 (10) or the buffer alone was added to the precleared cell lysate. The immune complex was then precipitated with anti-human IgM-biotin and Streptavidin-agarose, and the precipitates were subjected to SDS-PAGE followed by autoradiography. Immunoprecipitates with HuMAb L92 showed a clear band at M, 43,000. However, the immunoprecipitate, either with HuMAb L612 or without primary antibody, failed to show the band (Fig. 2), indicating that the M, 43,000 protein band in lane 3 specifically binds to HuMAb L92.

Screening of Immunopositive Clone(s) from M14 Cell Line cDNA Expression Library using HuMAb L92. To isolate the cDNA clones that encode the HuMAb L92 immunopositive protein, we constructed a cDNA expression library from M14 cells using the Agt11 recombinant phage system. We screened approximately 6 × 106 phages and obtained 2 positive clones, one of which was chosen for subsequent analysis.

Expression of Immunopositive Protein in E. coli. To determine the antigenicity of the cloned cDNA, we expressed the cDNA as a fused protein with β-galactosidase in the E. coli lysogen system and analyzed the recombinant protein by Western blot (Fig. 3). HuMAb L92 reacted with a band at M, 106,000 in the Western blot (lane 1).

![Western Blot Analysis](Fig. 1) SDS-PAGE and Western blot analyses of melanoma cell lysates. Lane 1, molecular weight standard (in thousands); lane 2, M14 human melanoma cell line grown in human AB serum; lane 3, M14 human melanoma cell line grown in medium containing fetal calf serum; lane 4, M12 human melanoma cell line grown in medium containing fetal calf serum; lane 5, fetal brain, second trimester. A, Western blot with HuMAb L92; B, Western blot with nonspecific human IgM.
The lysate obtained from a control experiment (without IPTG induction) did not show this band (lane 2). In lane 3, β-galactosidase shows a clear band in Coomassie Brilliant Blue staining; however, this band does not bind HuMAb L92. These data indicate that the amino acids derived from cDNA of the β-galactosidase fusion protein have antigenicity against HuMAb L92.

Nucleotide Sequence and Deduced Amino Acid Sequence of Immunopositive Clone. To characterize the cloned DNA, we first attempted to subclone the insert cDNA into the EcoRI site of pBlue-script II (Stratagene). However, we failed to isolate the insert cDNA with EcoRI digestion. To determine the cDNA sequence, we used whole phage DNA as a template. We used the Double Stranded DNA Cycle Sequencing System and obtained a partial sequence of the insert DNA of this clone is 1.4 kilobase pairs. We searched the homology between the open reading frame sequence and the reported cDNA sequences using PC-Gene software; however, no significant homology was obtained.

**HuMAb L92 Recognizes the Amino Acid Sequence Deduced from Isolated Clones.** We synthesized an oligopeptide based on the deduced amino acid sequence of clone 810 and tested it for its ability to inhibit HuMAb L92 binding to recombinant fused protein and M14 cell lysates. HuMAb L92 was preincubated with a 500-fold molar excess of a 14-amino acid peptide (DSRPQDLMKYQIF). This peptide sequence contains not only the sequence derived from cDNA but also the sequence derived from the EcoRI adaptor. HuMAb L92 without preincubation with the peptide made a discrete band with fused protein and M14 cell extracts at Mr 106,000 and 43,000, respectively (Fig. 5A). However, HuMAb L92 preincubated with peptide showed a significant reduction in the reactivity and showed only a faint band on the Western blot (Fig. 5B).

We also tested the 10-amino acid peptide (QDLTMKYQIF) after deleting the adaptor-derived sequence from the 14-amino acid peptide, which showed similar binding inhibition of HuMAb L92 in Western blot analysis (data not shown). Direct reactivity of HuMAb L92 to the 10-amino acid peptide was also examined by ELISA. A very strong and specific reactivity was demonstrated using 1 µg peptide/well. On the other hand, a control HuMAb L612 was completely negative, even at 10 µg/well peptide (Fig. 6). These results indicate that the 10-amino acid oligopeptide has antigenicity against HuMAb L92.

To further determine the minimum amino acids required for the antigenic epitope of HuMAb L92, several GST-fused proteins that were truncated from the cDNA peptide sequence (no. 810) were prepared using synthetic oligonucleotides and pGEX-2T vector. While the truncation of 1-amino acid residue from the COOH terminus did not affect the binding of HuMAb L92 (Fig. 7, lane 3), that of 2-amino acid residues completely diminished the antibody binding (lane 4). The deletion of 6-amino acid residues from the NH2 terminus of the 10-amino acid peptides did not affect the binding of HuMAb L92 (lane 7), but that of the 7-amino acid residues diminished the antibody binding (lane 8). These results demonstrate that the minimum antigenic epitope of HuMAb L92 is a 4-amino acid peptide (Lys-Tyr-Gln-Ile).

**Localization of Mr. 43,000 Protein in Melanoma Cells.** To determine whether the Mr. 43,000 protein is exposed on the cell surface or is located inside the cell, HuMAb L92 was absorbed with intact M14 cells and its reactivity to recombinant fused protein and Mr. 43,000 protein was tested by Western blot analysis. The staining intensities of the Mr. 43,000 protein on the cell surface varied widely among cell lines; for example, UCLASO M25 cell line (11) expressed the highest degree of the Mr 43,000 protein on the cell surface among 20 human melanoma cell lines tested.

**DISCUSSION**

HuMAb L92 was used to identify and clone a gene encoding the immunoreactive epitope in an M, 43,000 protein present in human melanoma. The epitope-encoding gene is composed of a newly dis-
Melanoma-associated antigens (AU and FD) unique to autologous melanoma cells have been detected using autologous sera (16). The similarity of the AU antigen to that studied in this report is not known. However, it is known that, although our $M_r 43,000$ antigen is shared by several cancer cell lines, the AU antigen is restricted to the autologous melanoma cell lines. The FD antigen is an $M_r 90,000$ cell surface glycoprotein, and the antigenic determinant is present on an ion-binding protein with homology to transferrin (17). The same molecule was identified by murine monoclonal antibodies developed against the human melanoma-associated antigen p97 (18). Although the sequence of p97 has been determined, that of the unique FD epitope has not yet been determined, and the present study did not discover a homologous sequence in the melanotransferrin with the 10-amino acid peptide sequence. Vlock et al. (19) used autologous melanoma sera after dissociating serum immune complexes via acidic treatment and found an antigenic $M_r 66,000$ acidic glycoprotein. Subsequent epitope analysis has shown that the carbohydrate moiety of the glycoprotein represents its antigenic determinant. The sequence of the core protein has not been reported.

Allogeneic polyclonal sera from melanoma patients have also been used to identify immunogenic melanoma-associated antigens. These antigens are shared by more than one melanoma. Li et al. (20) used sera from patients who received immunotherapy with melanoma cell supernatant to detect immunogenic melanoma-associated antigens ($M_r 200,000$, $150,000$, $110,000$, $75,000$, and $38,000$) (20). The obvious difference between our $M_r 43,000$ antigen and these antigens is their molecular weights. Euhus et al. (21) have defined a urinary tumor-associated antigen, which is a glycoprotein antigen originally found in the urine of melanoma patients and also found on the melanoma cell surface, including the M14 cell line. The urinary tumor-associated antigen is comprised of several subunits that are linked together by disulfide bonds. The total molecular weight is approximately 300,000, with immunogenic subunits of 45,000, 65,000, 90,000, 120,000, and 150,000 (21). None of these subunits bear the same antigen since HuMAb L92 does not react either to the $M_r 300,000$ protein or to the subunits. Hayashibe et al. used pooled sera from melanoma patients to identify an $M_r 50,000$ glycoprotein antigen (D-1) in melanoma (22). The immunoreactive clone was isolated and sequenced using cDNA libraries of a melanoma cell line. The successful cloning was possible after extensive absorption of non-specific antibodies with E. coli proteins and by blocking of cDNA plaques on nitrocellulose with IgG isolated from the pooled sera of healthy donors. While the exact tumor specificity of this protein (D-1) is not known, Northern blot hybridization has shown that PBL and normal fibroblasts do not express this antigen.

In summary, none of these melanoma-associated autoimmunogenic antigens appears to be the same as that examined in the present study. However, several other studies have identified protein molecules in

covered 4-amino acid sequence, which is expressed in the form of a fused protein with β-galactosidase or glutathione S-transferase in E. coli. HuMAb specifically bound to both the fused protein and the synthetic peptide. Absorption of HuMAb L92 with melanoma cells has proven that the antigen is an intracellular molecule in certain melanomas, while a high density of the antigen is expressed on the cell surface of other melanomas. Although immunological assays used to detect surface antigens revealed that HuMAb L92 did not react with normal cells, SDS-PAGE-Western blot analysis clearly revealed a positive band with normal tissue extracts at the $M_r 43,000$ position. Further investigation is required to determine an accurate distribution of the antigen epitope in various human cells and its potential utility as a vaccine for cancer treatment.

Research has found that melanoma has several protein, glycoprotein, and glycolipid antigens that elicit immune responses in man and may be useful as cancer vaccines. The best characterized autoimmune antigens of melanoma are gangliosides. Gangliosides are glycolipid molecules that exist predominantly on the cell surface. Human polyclonal and monoclonal antibodies have identified gangliosides $G_{D2}$, $G_{D2}$, and 9-O-acetylated $G_{D2}$ on human melanoma (4, 5, 12). These antigens are considered to be T-cell-independent antigens because immune responses against gangliosides are predominantly of IgM class, and no cellular responses have been detected. Nevertheless, it has been demonstrated that titers of IgM antibodies to these antigens are associated with prolonged survival of melanoma patients (13-15). In the present study, experiments were designed to identify an immunogenic protein antigen using a human monoclonal antibody. The objective of our study is to produce peptide antigens that induce cellular responses to cancer in man.
other human cancer cells that migrate at or around the $M_i$ 43,000 position in SDS-PAGE Western blot analysis. These studies used murine or human monoclonal antibodies. HGP43 is a human glycoprotein of $M_i$ 43,000, which was identified by a murine monoclonal antibody and was originally reported as a protective antibody against lethal *Listeria monocytogenes* infection in mice (23). HGP43 is detected in the urine of healthy, normal individuals as well as in cancer patients. Interestingly, HGP43 could stimulate mouse monocytes to induce cytotoxicity against the Lewis lung tumor (24). Both studies (23, 24) appear to have determined the amino acid sequence reactive to the monoclonal antibody, but neither reference revealed it. The human monoclonal antibodies 16.88 and C-OU 1 have identified another $M_i$ 43,000 protein molecule in human cancer cells (25). The antigen is most strongly expressed in melanoma, followed by colon cancer cells. The amino acid sequence so far established shows about 70% identity with cytokeratin 18. The 10-aminoo acid sequence reactive to our HuMAb L92 did not match any portion of the amino acid sequence of cytokeratin 18, indicating that these two antibodies recognize different molecules or epitopes.

Another HuMAb, MS2B6, has detected a cytoplasmic antigen present in a high density in 10% of ovarian carcinoma and less prevalent in a variety of human cancers, as well as in certain types of normal tissues (26, 27). On SDS-PAGE Western blot analysis, MS2B6 reacted to proteins with a broad range of molecular weights, 33,000—44,000 and 60,000. The competitive inhibition studies have shown no cross-reactivity between the antigen and the cytokeratin. The similarity of HuMAb L92 to MS2B6 is not known, but the immunoreactive band with HuMAb L92 on Western blotting has produced a sharp and clear band of $M_i$ 43,000, indicating that HuMAb L92 detects a different antigenic epitope than does MS2B6. Since the papers did not report amino acid sequences of any of these $M_i$ 43,000- or 43,000-area proteins, exact comparison of these is not possible. The possibility that these antigens detect different epitopes on the same molecule has not been excluded. This will be clarified when a full length of amino acid sequence of the protein(s) is determined.

Although our efforts to clone and evaluate the antigen are still very preliminary, the significance of this study includes the following: (a) human monoclonal antibody may be a more efficient reagent for gene cloning of tumor-associated molecules immunogenic in man. An antibody-reactive peptide in the $M_i$ 43,000 protein was specifically identified; (b) the sequence of cDNA cloned and selected by HuMAb may be useful as a primer or a probe to isolate the full length of the $M_i$ 43,000 protein. Full-length peptide analysis is important to determine the location of the antigen epitope, to search other sites for a gene clone of monoclonal antibodies; and finally (d) the peptide itself may be clinically very useful as a primer or a probe to isolate the full length of the $M_i$ 43,000 protein of which was identified by a murine monoclonal antibody in metastatic melanoma patients after vaccination with melanoma-associated antigens. Int. J. Cancer, 64: 607—612, 1985.


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