Characterization and Immunotherapeutic Implications for a Novel Antibody Targeting Interleukin (IL)-13 Receptor α2

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**Background:** Antibodies specific for tumor-associated antigens (TAAs) have emerged as valuable research, diagnostic, and therapeutic agents.

**Results:** A novel antibody against TAA IL13Ra2 has been generated and characterized.

**Conclusion:** The antibody possesses a high specificity and affinity for IL13Ra2 and competes with IL-13 for binding to IL13Ra2.

**Significance:** Future studies testing the therapeutic and diagnostic properties of this antibody in IL13Ra2-expressing tumors are now possible.

The high affinity interleukin-13 receptor α2 (IL13Ra2) is selectively expressed at a high frequency by glioblastoma multiforme (GBM) as well as several other tumor types. One approach for targeting this tumor-specific receptor utilizes the cognate ligand, IL-13, conjugated to cytotoxic molecules. However, this approach lacks specificity because the lower affinity receptor for IL-13, IL13Ra1, is widely expressed by normal tissues. Here, we aimed to develop and characterize a novel monoclonal antibody (mAb) specific to IL13Ra2 for the therapeutic purpose of targeting IL13Ra2-expressing tumors. Hybridoma cell lines were generated and compared for binding affinities to recombinant human IL13Ra2 (rhIL13Ra2). Clone 47 demonstrated binding to the native conformation of IL13Ra2 and was therefore chosen for further studies. Clone 47 bound specifically and with high affinity (K_D ≈ 1.39 × 10^{-7} M) to rhIL13Ra2 but not to rhIL13Ra1 or murine IL13Ra2. Furthermore, clone 47 specifically recognized wild-type IL13Ra2 expressed on the surface of CHO and HEK cells as well as several glioma cell lines. Competitive binding assays revealed that clone 47 also significantly inhibited the interaction between human soluble IL-13 and IL13Ra2 receptor. Moreover, we found that N-linked glycosylation of IL13Ra2 contributes in part to the interaction of the antibody to IL13Ra2. In vivo, the IL13Ra2 mAb improved the survival of nude mice intracranially implanted with a human U251 glioma xenograft. Collectively, these data warrant further investigation of this novel IL13Ra2 mAb with an emphasis on translational implications for therapeutic use.

Proteins expressed by tumor cells but not by normal cells are attractive molecules for the selective delivery of cytotoxic molecules. Accordingly, interleukin-13 receptor α2 (IL13Ra2), the high affinity receptor for interleukin-13 (IL-13), is a promising candidate. IL13Ra2 is expressed at a high frequency in the aggressive and incurable form of primary brain tumor known as glioblastoma multiforme (GBM) (1–3) as well as by other solid tumors (4). In contrast, normal tissues express little to no IL13Ra2 with the exception of the testes (6). Notably, IL13Ra1, a different receptor with low affinity for IL-13, is expressed ubiquitously by many tissues (7–9), making it a poor candidate for selective targeting of tumor-specific immunotherapeutic applications.

Until recently, IL13Ra2 was thought to act as a decoy receptor for IL-13 (10). However, recent studies have challenged that theory based on studies demonstrating that upon binding of IL-13 to IL13Ra2 downstream signaling occurs in specialized pulmonary macrophages (11) as well as in pancreatic ductal (12) and ovarian carcinoma cells (13). Moreover, overexpression of IL13Ra2 in GBM but not in normal brain tissue (14, 15) uniquely positions this receptor as a candidate for targeting tumor cells. GBM is a highly infiltrative tumor often making complete surgical removal impossible. Moreover, GBM is highly resistant to radiation and chemotherapy (16), warranting further development of novel and targeted therapies for the treatment of patients. Several studies have investigated the therapeutic properties of an IL-13 fusion protein conjugated to a recombinant cytotoxin derived from *Pseudomonas exotoxin A* (IL-13PE) that induces apoptosis in IL13Ra2-expressing glioma cells *in vitro*, in preclinical animal models, and in patients tested in clinical trials (17–22). However, such agents lack a high specificity of interaction with IL13Ra2 due to the alternative binding of ubiquitously expressed IL13Ra1. Therefore, developing highly selective antibody fragments that can be combined with toxins for specificity to IL13Ra2-expressing cells is considered to be a promising pursuit.

Previous work has investigated a phage display library approach for selecting small antibody fragments specific to IL-13Ra2. The abbreviations used are: IL13Ra1, interleukin-13 receptor; GBM, glioblastoma multiforme; rh, recombinant human; Pngase F, peptide-N-glycosidase F; SPR, surface plasmon resonance; RU, response units; hFc, human Fc; sc, single chain; mIgG, mouse IgG.
human IL13Ra2 followed by their evaluation in vitro and in vivo (23). Despite the high specificity of interaction with IL13Ra2, conjugation with toxins has failed to increase cytotoxicity in IL13Ra2-expressing glioma and renal cell carcinoma cell lines when compared with the effects of IL-13PE38. The low affinity of generated antibody fragments is the most reasonable explanation for the lack of success. Antibody fragments derived from phage display libraries are known to be lower in affinity and avidity than antibodies generated by conventional hybridoma technology (24). Modifications of those small antibody fragments are often required to enhance their affinity and avidity to targeted proteins. In recent years, monoclonal antibodies have shown increasing success as targeted anticancer and diagnostic agents (25, 26), and a further search for high affinity reagents with restricted specificity to tumor-associated antigens is in progress. Historically, the hybridoma cell line specific to the antigen IL13Ra2, however, has been unavailable to the scientific community. Thus, the goal of the present study was to discover, develop, and characterize a high affinity antibody that specifically recognizes IL13Ra2 expressed on the surface of cancer cells. Here, we demonstrate the generation of an antibody possessing the properties critical for immunotherapeutic targeting of IL13Ra2-expressing tumors in vivo and potentially suitable for various other applications.

**EXPERIMENTAL PROCEDURES**

Materials—Lipofectamine 2000 and the pEF6/Myc–His vector were obtained from Invitrogen. mAbs to IL13Ra2 (clones YY-23Z and B-D13) and the IsoStrip mouse monoclonal antibody isotyping kit were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The mAb to IL13Ra2 (clone 83807) and recombinant human and mouse IL13Ra2hFc and IL13Ra1hFc chimeras were purchased from R&D Systems (Minneapolis, MN). Biotinylated horse anti-mouse antibodies and the Elite kit were obtained from Vector Laboratories (Burlingame, CA). 3,3′-Diaminobenzidine substrate was purchased from Dako (Carpinteria, CA). Goat anti-mouse antibody conjugated with peroxidase was purchased from Chemicon International (Temecula, CA), and Pnase F was purchased from New England Biolabs (Ipswich, MA). The QuikChange Lightning site-directed mutagenesis kit was purchased from Agilent Technologies, Inc. (Santa Clara, CA), and the RNaseasy Plus kit was received from Qiagen (Valencia, CA). The cDNA iScript kit, 7.5% Tris-HCl gel, and ImmunStar WesternC developing reagent and protein marker were purchased from Bio-Rad. The human IL-13 ELISA kit was purchased from eBioscience (San Diego, CA). GBM12 and GBM43 were kindly provided by Dr. David C. James (University of California-San Francisco), and the cDNA encoding human wild-type IL13Ra2 was obtained from Dr. Waldemar Debinski (Wake Forest University).

Immunization—To obtain monoclonal antibodies with specificity to native IL13Ra2, the human recombinant IL13Ra2hFc fusion was used for immunization of animals and in all screening assays. Two 6-week-old female BALB/c mice were immunized with intraperitoneal injection of 10 μg of rhIL13Ra2hFc protein in complete Freund’s adjuvant followed by intraperitoneal injection of 10 μg of rhIL13Ra2hFc protein in incomplete Freund’s adjuvant at a 2-week interval for 2 months. Two weeks after the last intraperitoneal injection and 3 days before the fusion, a boost was performed by the combination of intravenous and intraperitoneal injection of 10 μg of antigen without Freund’s adjuvant. The fusion of mouse spleen cells with the mouse myeloma cell line X63.Ag8.653 subclone P3O1 was performed by using a procedure described by Köhler and Milstein (27). Hybridoma supernatants were assayed for the presence of IL13Ra2 antibodies using the enzyme-linked immunosorbent assay (ELISA). Selected populations were cloned, and supernatants were assayed to identify the clones with strongest binding.

Generation of CHO Cell Line Expressing Human IL13Ra2—The cDNA encoding human wild-type IL13Ra2 was amplified with the following primer pair: forward, 5′-GCTTGGTACCCGAATGCTTTTCGTTGGC-3′ and reverse, 5′-GGTTTTGTTCCGAATGTTACACAGAAGAAATCTGG-3′. The purified PCR product was restricted with Kpn1 and BstBI enzymes, agarose gel-purified, and subsequently cloned into the pEF6/Myc–His vector in a reading frame with Myc and His_6 tags. CHO cells were plated at 80% confluence and transfected with a plasmid encoding the IL13Ra2 using Lipofectamine 2000. The following day, 4 μg/ml blasticidin was added for selection of cells that had stably incorporated and expressed the IL13Ra2 transcript. A stable population of cells was further subcloned in 96-well plates at a density of one cell/well. Ten days later, single clones were screened by flow cytometry for cell surface expression of IL13Ra2 using an antibody to IL13Ra2 (clone B-D13). The clone with the highest level of IL13Ra2 expression was selected and expanded for subsequent screening of hybridomas secreting IL13Ra2 antibodies.

ELISA—96-well plates were coated with 50 μl of human or mouse recombinant IL13Ra2hFc or IL13Ra1hFc or human control IgG at a concentration of 1 μg/ml overnight at 4 °C. Following washes with TBS-Tween 20 buffer and blocking with 1% nonfat dry milk, 50 μl of purified antibodies, serum, or hybridoma supernatants at various dilutions were applied to the plate and incubated for 1 h at room temperature. Bound antibodies were detected with goat anti-mouse antibodies conjugated to alkaline phosphatase following the development with alkaline phosphatase substrate. Plates were read at A_405 using a UniRead 800 plate reader (BioTek).

Flow Cytometry—CHO or HEK cells expressing IL13Ra2; the glioma cell lines A172, N10, U251, U87, and U118; patient-derived GBM12 and GBM43, and primary human astrocytes were stained with IL13Ra2 (clone 47) mAb at a 1 μg/ml followed by goat anti-mouse Alexa Fluor 647 (1:500). All staining procedures were performed on ice. Samples were analyzed using the BD FACSCanto flow cytometer and FACSDiVa™ software.

PCR—to determine the expression of IL13Ra2 in various glioma cells and astrocytes, total RNA was generated from the cell pellets using the RNeasy Plus kit. 200 ng of total RNA was then converted into cDNA using the cDNA iScript kit. The cDNA was further amplified for IL13Ra2 and GAPDH for 30 cycles using IL13Ra2 and GAPDH primers and visualized on a 1% agarose gel.

Surface Plasmon Resonance—The affinity and rates of interaction between IL13Ra2 (clone 47) mAb, commercially available IL13Ra2 mAbs (clones 83807 and B-D13), and target (rhIL13Ra2) were measured with a Biacore 3000 biosensor.
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through surface plasmon resonance (SPR). The mAbs were immobilized (covalently) to the dextran matrix of the sensor chip (CM5) using the amino coupling kit. The carboxyl groups on the sensor surfaces were activated with an injection of a solution containing 0.2 mM N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide and 0.05 mM N-hydroxysuccinimide. The immobilization procedure was completed by the injection of 1 mM ethanalamine hydrochloride to block the remaining ester groups. All steps of the immobilization process were carried out at a flow rate of 10 µl/min. The control surface was prepared similarly with the exception that running buffer was injected rather than mAbs. Binding reactions were performed at 25 °C in HBS-P buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, and 0.005% (v/v) surfactant P20) using a flow rate of 20 µl/min. Target (rhIL13Ra2) was added at various concentrations in the flow during the binding phase. The amount of protein bound to the sensor chip was monitored by the change in refractive index (represented by response units (RU)). The instrument was programmed to perform a series of binding measurements with increasing concentrations of target over the same surface. Triplicate injections of each concentration of target were performed. Sensorgrams (plots of changes in RU on the surface as a function of time) were analyzed using BIAevaluation v4.1. Affinity constants were estimated by curve fitting using a 1:1 binding model.

Data Preparation and Kinetic Analysis—The estimation of kinetic parameters was performed by repetitive injections of a range of target concentrations over the immobilized mAbs. Data were prepared by the method of “double referencing.” This method utilizes parallel injections of each target sample over a control dextran surface as well as running buffer injections over both the immobilized mAbs and control dextran surfaces. Subtraction of these sensorgrams yielded the control; this was subtracted from the experimental sensorgram. Each data set (consisting of sensorgrams of increasing target concentrations over the same level of immobilized mAbs) was analyzed using various kinetic models. The BIAevaluation v 4.1 software was then used for data analysis. Affinity constants were estimated by curve fitting using a 1:1 binding model. Sensorgram association and dissociation curves were fit locally or globally. The rate of complex formation during the sample injection is described by an equation of the following type: \( dR/dt = k_aC(R_{\text{max}} - R) - k_dR \) (for a 1:1 interaction) where \( R \) is the SPR signal in RU, \( C \) is the concentration of analyte, \( R_{\text{max}} \) is the maximum analyte binding capacity in RU, and \( dR/dt \) is the rate of change of SPR signal. The early binding phase (300 s) was used to determine the association constant \( (k_a) \) between mAb and target. The dissociation phase \( (k_d) \) was measured using the rate of decline in RU on introduction of free buffer at the end of target injections. Data were simultaneously fit by the software program (global fitting algorithm), and the dissociation constant \( (K_d) \) of the complexes was determined as the ratio \( k_d/k_a \). For quantitative analysis, three independent replicates were performed for each sample. Data are expressed as mean ± S.E.

Competitive Binding Assay—For the competitive binding plate assay, a 96-well plate was coated with 50 µl of affinity-purified hIL13Ra2Fc at 1 µg/ml in carbonate buffer, pH 9.6 and stored overnight at 4 °C. After washing with PBS containing 0.05% Tween 20, mAbs to IL13Ra2 (10 µg/ml) or control mlgG were added for 30 min at room temperature. After washing, 50 µl of purified rhIL-13 in PBS and 0.1% BSA at 10 ng/ml was added for a 1-h incubation at room temperature and assayed for bound rhIL-13 using detection reagents from a human IL-13 ELISA kit. Separately, HEK cells expressing wild-type or 4-amino acid mutants in the IL13Ra2 sequence were pretreated with either rhIL-13 or mAb IL13Ra2 (clone 47) at 2 µg/ml for 30 min on ice followed by a 1-h incubation with IL13Ra2 (clone 47) mAb or rhIL-13 at 100 ng/ml, respectively. Binding of rhIL-13 to IL13Ra2 alone or in presence of the competitor was detected with human IL-13 mAb-FITC. Binding of IL13Ra2 (clone 47) mAb to rhIL13Ra2 alone or in presence of the competitor was detected with anti-mouse antibody conjugated to Alexa Fluor 649 and analyzed by flow cytometry.

Mutagenesis of IL13Ra—Previously, Tyr207, Asp271, Tyr315, and Asp318 of the human IL13Ra2 were identified as residues crucial for the interaction with human IL-13 (28). To determine whether these residues were important for binding of the IL13Ra2 (clone 47) mAb to IL13Ra2, the Tyr207, Asp271, Tyr315, and Asp318 were mutated to Ala separately or at the same time (4-amino acid mutant) using the QuickChange Site-directed mutagenesis kit according to the manufacturer’s recommendations. Sequencing of selected clones was performed in house and confirmed the presence of the selected mutation. HEK cells were transfected with wild-type or mutated variants of IL13Ra2 cDNA in the pEF6 Myc-His vector using Lipofectamine Plus transfection reagent. 48 h after transfection, the cells were collected and analyzed for binding to IL13Ra2 (clone 47) mAb via flow cytometry.

Western Blot—The rhIL13Ra2 was applied to a 7.5% Tris-HCl gel (Bio-Rad) at 200 ng/lane and resolved under reducing conditions. After the transfer of proteins to a PVDF membrane (Bio-Rad) and blocking with 2% nonfat dry milk, the membrane was stained with anti-IL13Ra2 mAb (clones YY-23Z and B-D13) at 2 μg/ml or with supernatant collected from hybridoma clones (diluted 10 times), followed by goat anti-mouse antibody conjugated to peroxidase. ImmunStar WesternC was used to develop the reaction. Images were captured using a Bio-Rad ChemiDoc imaging system.

Immunohistochemistry—The GBM tissues were collected in accordance with a protocol approved by the Institutional Review Board at the University of Chicago. Flash frozen brain tumor tissues were cut to a thickness of 10 µm. Tissue sections were fixed with −20 °C methanol and stained for human IL13Ra2 using mouse IL13Ra2 (clone 47) mAb at a concentration of 3 µg/ml or isotype control mlgG1. The bound antibodies were detected with biotinylated horse anti-mouse antibodies (1:100). The antigen-antibody binding was detected by the Elite kit with 3,3'-diaminobenzidine substrate. Slides were analyzed using the CRI Panoramic Scan Whole Slide Scanner and Panoramic Viewer software.

Animal Study—All animals were maintained and cared for in accordance with the Institutional Animal Care and Use Committee protocol and according to National Institutes of Health guidelines. The animals used in the experiments were 6–7 week-old male athymic nu/nu mice. Mice were anesthetized with an intraperitoneal injection of ketamine hydrochloride/
xylazine (25 mg/ml/2.5 mg/ml) mixture. To establish intracranial tumors, a midline cranial incision was made, and a rightsided burr hole was placed 2 mm lateral to the sagittal suture and 2 mm superior to it. Animals were positioned in a stereotactic frame, and a Hamilton needle was inserted through the burr hole and advanced 3 mm. Intracranial penetration was followed by (i) injection of 2.5 × 10⁴ U251 glioma cells in 2.5 ml of sterile PBS in combination with 200 ng of mIgG or IL13Rα2 (clone 47) mAb or (ii) 3 days postintracranial injection of glioma cells with PBS or 10 g of IL13Rα2 (clone 47 or B-D13) mAb as described previously (29). All mice were monitored for survival. Three animals from each group were sacrificed at day 17, and brains were harvested and frozen for sectioning, hematoxylin and eosin (H&E) staining, and microscopic analysis.

Statistics—The differences between groups were evaluated by Student’s t test or one-way analysis of variance with post hoc comparison Tukey’s test or Dunnett’s test. For the in vivo survival data, a Kaplan-Meier survival analysis was used, and statistical analysis was performed using a log rank test. p < 0.05 was considered statistically significant.

RESULTS

Characterization of Antigen and Screening of Hybridoma Clones Secreting Anti-IL13Rα2 Antibodies—The primary goal of this study was to generate a high affinity monoclonal antibody suitable for targeting of the IL13Rα2 expressed on the surface of tumor cells. We therefore immunized mice and screened the resulting hybridoma clones for reactivity against the antigen, rhIL13Rα2, in its native conformation. A plate-bound ELISA utilizing a hybridoma clone against rhIL13Rα2, YY-23Z, was established for the detection of rhIL13Rα2. The concentration of rhIL13Rα2 absorbed to the plastic at 1 μg/ml was found to be suitable for the detection of antibody binding (Fig. 1A). Next, the rhIL13Rα2hFc was characterized for its “nativity” by utilizing a pair of commercially available antibodies recognizing only the native (found on the cell surface) and denatured (using Western blotting under reducing conditions) forms of IL13Rα2 and for its binding properties to rhIL13Rα2 in ELISA with antibody clones B-D13 and YY-23Z, respectively. Both clones B-D13 and YY-23Z were able to recognize the rhIL13Rα2hFc in a plate-bound ELISA (Fig. 1B). Denaturation of antigen at 95 °C for 5 min in the presence of β-mercaptoethanol completely abolished the ability of the antibody clone B-D13 to recognize antigen by ELISA, whereas the YY-23Z clone retained the ability to bind the denatured antigen. Thus, the rhIL13Rα2hFc absorbed to the plastic of ELISA plates containing both native and denatured forms of the protein. Analysis of serum from animals immunized with a fusion of rhIL13Rα2 and hFc revealed the presence of antibodies against both rhIL13Rα2 and human Fc fragment (data not shown). To select antibodies specific for the IL13Rα2 portion of the fusion, human IgG was included as an additional negative control for the screening of hybridoma populations against rhIL13Rα2hFc in a plate-bound ELISA. D, screening of selected hybridoma populations against rhIL13Rα2hFc using a Western blot.
clones strongly reacting with native IL13Rα2 were further expanded and recloned. The two clones recognizing only denatured antigen were selected from the separate immunization set with rhIL13Rα2hFc chimera (data not shown). Supernatants from selected clones were compared for their ability to bind hrIL13Rα2 in a plate-bound ELISA (Fig. 1C) and by Western blotting (Fig. 1D). Fig. 1C shows that clone 47 strongly binds to the antigen in plate-bound ELISA but not by Western blotting, indicating the ability of clone 47 to recognize a native conformation of the antigen. Therefore, clone 47 was selected for all subsequent studies and analysis for its properties. Clone 47 was found to be of the IgG1 isotype possessing a IgG1 chain (data not shown).

Specificity of Binding for the IL13Rα2 (Clone 47) mAb to Recombinant Human IL13Rα2 and IL13Rα2 Expressed at the Cell Surface—We investigated the binding properties of the IL13Rα2 (clone 47) mAb to rhIL13Rα2 versus the commercially available clones 83807 and B-D13 in a plate-bound ELISA. Fig. 2A shows strong and specific binding of clone 47 to rhIL13Rα2 when compared with clones 83807 and B-D13. Clone 47 reaches the plateau of binding at the low concentration of 0.05 μg/ml. None of the antibodies showed binding to human IgG utilized as an additional negative control in these experiments (data not shown).

To further verify the specificity of interaction for clone 47 with human IL13Rα2, a clonal line of CHO cells expressing the full size wild-type human IL13Rα2 (clone 6) was generated. Binding of the antibody to control CHO cells transfected with an empty vector was compared with that of CHO cells expressing IL13Rα2. Again, the IL13Rα2 (clone 47) mAb demonstrated strong and specific binding to IL13Rα2 expressed on the cell surface but not to control CHO cells, indicating that this antibody specifically recognizes a native conformation of the IL13Rα2 (Fig. 2B). Clone 47 demonstrated the strongest affinity for IL13Rα2 at the lowest tested concentration of 0.25 μg/ml. Notably, other selected hybridoma clones demonstrated similar specificity of interaction with IL13Rα2 expressed on the cell surface of CHO cells but not with control CHO cells (data not shown). Data obtained in a plate-bound ELISA also revealed that clone 47 does not interact with the low affinity receptor for IL-13, the IL13Rα1 (Fig. 2C), or mouse recombinant IL13Rα2, further validating the specificity of interaction between clone 47 and IL13Rα2 (Fig. 2D). Clones 83807 and B-D13 did not show binding to mouse rIL13Rα2 in agreement with current understanding of the cross-reactivity of these antibodies with mouse IL13Rα2.

We next characterized the binding capacity of clone 47 with various glioma cell lines, the patient-derived glioma lines GBM12 and GBM43, and normal human astrocytes. Increased expression of the IL13Rα2 gene relative to normal brain tissue is reported in 44–47% of human GBM resected specimens (3) and in up to 82% (14 of 17) primary cell cultures derived from GBM and normal brain explants (2). Fig. 3, A and B, show the flow charts of the comparative staining of glioma cells, human astrocytes, and HEK cells expressing recombinant human IL13Rα2 on the cell surface with the IL13Rα2 (clones 47, 83807, and B-D13) mAbs. Fig. 3, A and B, reveal (i) various levels of IL13Rα2 expression on the cell surface and (ii) superior binding...
of the clone 47 versus clones B-D13 (1.2–4.6-fold difference between the cell lines) and 83807 to the surface of analyzed cell lines. Interestingly, we observed a near complete absence of the binding of clone 83807 to glioma cell lines in contrast to HEK cells expressing IL13Rα2. No binding of clone 47 was detected with normal human astrocytes, confirming the specificity of interaction of clone 47 with human glioma cells expressing IL13Rα2. The expression of IL13Rα2 mRNA in these cells generally correlates with the level of IL13Rα2 expression on the cell surface. Moreover, cells expressing low to no mRNA expression for IL13Rα2, including U118 and primary human astrocytes, demonstrated low to no expression for IL13Rα2 on the cell surface (Fig. 3B). In additional experiments, N10 glioma cells were incubated with either the IL13Rα2 (clone 47) mAb at 1 μg/ml or the IL13Rα2 (clone 47) mAb preincubated with a 10× excess of rhIL13Rα2 (supplemental Fig. 1A) and analyzed by flow cytometry. A significant ablation of interaction between the IL13Rα2 (clone 47) mAb in the presence of a 10× excess of rhIL-13 or IL13Rα2 (clone 47) mAb almost completely blocked the interaction between the antibody or rhIL-13 and N10 cells (supplemental Fig. 1B), indicating a specificity of recognition between IL13Rα2 expressed on the surface of glioma cells and clone 47 (supplemental Fig. 1).

To verify that the IL13Rα2 (clone 47) mAb possessed the ability to bind IL13Rα2 on the surface of glioma cells in situ, intracranial glioma xenografts of U251 cells expressing green fluorescent protein (GFP) were established in nude mice. Three weeks later, animals were sacrificed, and cells were obtained and placed into in vitro culture conditions. After 48 h, the cells were collected and stained with control mIgG or IL13Rα2 (clone 47) mAb. Cultured GFP-expressing U251 cells served as a positive control (data not shown). GFP-positive U251 cells represented ~56% of the total cells (Fig. 3C, panel a), and 96% of the cells were reactive with the IL13Rα2 (clone 47) mAb (Fig. 3C, panel c), whereas GFP-negative cells did not interact with the antibody (Fig. 3C, panel b). These data further confirm that the IL13Rα2 (clone 47) mAb specifically recognizes glioma cells expressing IL13Rα2 in mouse xeno-
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To further verify the inhibitory properties of the IL13Ra2 (clone 47) mAb, HEK 293T cells were transfected with a construct encoding wild-type or a 4-amino acid mutant form of IL13Ra2 cDNA in which Tyr207, Asp271, Tyr315, and Asp318 residues were substituted to Ala. Previously, these residues of the human IL13Ra2 were identified as amino acids required for the interaction with the cognate ligand, IL-13. The presence of all four mutations in one molecule has been shown to result in near complete ablation of the binding of IL-13 to the mutated form of IL13Ra2 (28). After 48 h, the cells were pretreated with a 20× excess of rhIL-13 or the IL13Ra2 (clone 47) mAb followed by incubation of the IL13Ra2 (clone 47) mAb or rhIL-13, respectively. Fig. 5B shows an ~50% binding inhibition of IL13Ra2 (clone 47) mAb by a 20× excess of rhIL-13 to wild-type (WT) IL13Ra2 but not to a 4-amino acid mutant form of IL13Ra2. A 20× excess of antibody abolished the binding of rhIL-13 to IL13Ra2 when expressed on the cell surface by 80%, which is similar to the result observed in plate ELISA. The residual binding of IL-13 to the 4-amino acid mutant form of IL13Ra2 was further decreased by an excess of the IL13Ra2 (clone 47) mAb (Fig. 5C). Collectively, these data suggest that the IL13Ra2 (clone 47) mAb specifically competes with rhIL-13 for the binding site on IL13Ra2. Also, these data suggest that the IL13Ra2 (clone 47) mAb and IL-13 have a significant overlap in their recognition site of the IL13Ra2 molecule.

A Novel Monoclonal Antibody Competes with rhIL-13 for Binding to IL13Ra2—To determine whether the IL13Ra2 (clone 47) mAb possesses inhibitory properties, competitive binding assays utilizing a rhIL13Ra2hFc chimera and HEK cells transiently expressing the human IL13Ra2 were performed. The competitive binding assay was set up in a plate-bound ELISA format. The rhIL13Ra2hFc absorbed to the plate served as the target antigen. To determine whether the IL13Ra2 mAb specifically inhibits the binding of IL-13 to rhIL13Ra2, plates were preincubated with a 100× excess of mlgG, the IL13Ra2 mAb (clone 47) mAb, or other IL13Ra2 mAb clones, including 83807, YY-23Z, and B-D13, followed by incubation with rhIL-13. Fig. 5A shows that the IL13Ra2 (clone 47) mAb significantly abolished the binding of rhIL-13 to rhIL13Ra2, whereas the IL13Ra2 mAb clones B-D13 and 83807 competed for binding of human IL-13 significantly less.

To determine the affinity and rate of interaction between the IL13Ra2 (clone 47) mAb and rhIL13Ra2. All measurements were done in comparison with two commercial antibodies against IL13Ra2, clones 83807 and B-D13. Fig. 4 shows the sensorgrams for each antibody. The measurements are summarized in Table 1. Table 4A shows that clone 47 demonstrates a prolonged and stable association with rhIL13Ra2 measured over a 30-min time frame, whereas clones 83807 (Fig. 4B) and B-D13 (Fig. 4C) dissociate relatively quickly. The affinity of binding for the IL13Ra2 (clone 47) mAb to rhIL13Ra2 was calculated at 1.39 × 10⁻⁹ M. This value exceeded the affinity of the commercially available antibody clones 83807 and B-D13 to rhIL13Ra2 by 75× and 33×, respectively. Clone 47 demonstrated the highest binding affinity (R_max) to rhIL13Ra2 at 390 RU when compared with 250 and 8–16 RU for clones 83807 and B-D13, respectively. These data indicate that the IL13Ra2 (clone 47) mAb possesses properties superior to clones 83807 and B-D13 as well as demonstrates a higher affinity toward rhIL13Ra2.

A Novel Monoclonal Antibody Competes with rhIL-13 for Binding to IL13Ra2—To determine whether the IL13Ra2 (clone 47) mAb possesses inhibitory properties, competitive binding assays utilizing a rhIL13Ra2hFc chimera and HEK cells transiently expressing the human IL13Ra2 were performed. The competitive binding assay was set up in a plate-bound ELISA format. The rhIL13Ra2hFc absorbed to the plate served as the target antigen. To determine whether the IL13Ra2 mAb specifically inhibits the binding of IL-13 to rhIL13Ra2, plates were preincubated with a 100× excess of mlgG, the IL13Ra2 mAb (clone 47) mAb, or other IL13Ra2 mAb clones, including 83807, YY-23Z, and B-D13, followed by incubation with rhIL-13. Fig. 5A shows that the IL13Ra2 (clone 47) mAb significantly abolished the binding of rhIL-13 to rhIL13Ra2, whereas the IL13Ra2 mAb clones B-D13 and 83807 competed for binding of human IL-13 significantly less.

To further verify the inhibitory properties of the IL13Ra2 (clone 47) mAb, HEK 293T cells were transfected with a construct encoding wild-type or a 4-amino acid mutant form of IL13Ra2 cDNA in which Tyr207, Asp271, Tyr315, and Asp318 residues were substituted to Ala. Previously, these residues of the human IL13Ra2 were identified as amino acids required for the interaction with the cognate ligand, IL-13. The presence of all four mutations in one molecule has been shown to result in near complete ablation of the binding of IL-13 to the mutated form of IL13Ra2 (28). After 48 h, the cells were pretreated with a 20× excess of rhIL-13 or the IL13Ra2 (clone 47) mAb followed by incubation of the IL13Ra2 (clone 47) mAb or rhIL-13, respectively. Fig. 5B shows an ~50% binding inhibition of IL13Ra2 (clone 47) mAb by a 20× excess of rhIL-13 to wild-type (WT) IL13Ra2 but not to a 4-amino acid mutant form of IL13Ra2. A 20× excess of antibody abolished the binding of rhIL-13 to IL13Ra2 when expressed on the cell surface by 80%, which is similar to the result observed in plate ELISA. The residual binding of IL-13 to the 4-amino acid mutant form of IL13Ra2 was further decreased by an excess of the IL13Ra2 (clone 47) mAb (Fig. 5C). Collectively, these data suggest that the IL13Ra2 (clone 47) mAb specifically competes with rhIL-13 for the binding site on IL13Ra2. Also, these data suggest that the IL13Ra2 (clone 47) mAb and IL-13 have a significant overlap in their recognition site of the IL13Ra2 molecule.

![Figure 4](image_url) The affinity between the IL13Ra2 (clone 47) mAb and rhIL13Ra2. The kinetics of interaction of IL13Ra2 (clone 47) mAb (A) and the commercially available mAb clones 83807 (B) and B-D13 (C) with rhIL13Ra2 as visualized by SPR in a Biacore 3000 are shown. The rhIL13Ra2 was injected at concentrations ranging from 1 to 100 nM (lower to upper curves) at a constant flow rate of 20 µl/min over immobilized IL13Ra2 and over a control dextran surface (these values were subtracted from the signal). The association and dissociation phases were monitored for 300 s by following the change in SPR signal (colored curves) given in RU. Black curves represent the fit of the data to a one-site binding model. For derived kinetic parameters, see Table 1. Lower panels show residuals from a one-site binding model, indicating an excellent fit.

| mAbs to IL13Ra2 | k_a | k_d | K_D | R_max |
|-----------------|-----|-----|-----|-------|
| Clone 47        | 9.06e4 ± 322 | 1.26e−4 ± 1.07e−6 | 1.39 × 10⁻⁶ | 590 |
| Clone 83807     | 2.33e4 ± 620 | 2.31e−3 ± 1.03e−5 | 104 × 10⁻⁶ | 250 |
| Clone B-D13     | 1.08e5 ± 5.71e3 | 4.99e−3 ± 1.45e−4 | 46.1 × 10⁻⁹ | 8–16 |

*TABLE 1 Kinetics of monoclonal antibodies binding to the human recombinant IL13Ra2*

The estimation of kinetic parameters was performed as described under “Experimental Procedures.” The dissociation constant (K_d) of the complexes was determined as the ratio k_d/k_a. For quantitative analysis, three independent replicates were performed for each sample. Data are expressed as mean ± S.E. These data demonstrate that the affinity of IL13Ra2 (clone 47) mAb to recombinant IL13Ra2 exceeds the affinity of commercially available mAb clones 83807 and B-D13 by 75× and 33×, respectively.
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Role of the Tyr<sup>207</sup>, Asp<sup>271</sup>, Tyr<sup>315</sup>, and Asp<sup>318</sup> Residues for IL13Rα2 (Clone 47) mAb Binding—Taking into consideration that IL-13 and the IL13Rα2 (clone 47) mAb can significantly compete with one other for binding of IL13Rα2, we determined whether the residues Tyr<sup>207</sup>, Asp<sup>271</sup>, Tyr<sup>315</sup>, and Asp<sup>318</sup> contributing to the interaction of IL-13 with IL13Rα2 (28) are also important for binding of the IL13Rα2 (clone 47) mAb to IL13Rα2. The plasmids encoding cDNA for IL13Rα2 carrying individual mutations of Tyr<sup>207</sup>, Asp<sup>271</sup>, Tyr<sup>315</sup>, or Asp<sup>318</sup> residues to Ala or a combination of all four mutations in one molecule were generated and transiently expressed in HEK cells. Binding of the IL13Rα2 (clone 47) mAb to wild-type and mutant forms of IL13Rα2 was analyzed by flow cytometry. The IL13Rα2 mAbs 83807 and B-D13 were used as reference antibodies to exclude a possible influence of variations in the level of expression of wild-type or mutated variants of IL13Rα2 on the surface of HEK cells (Fig. 6A). Data were calculated as a ratio of IL13Rα2 (clone 47) binding to IL13Rα2 when compared with both antibody clones 83807 and B-D13. Fig. 6A demonstrates that the binding of IL13Rα2 (clone 47) mAb was not significantly affected by either the individual mutations or the 4-amino acid mutant form of IL13Rα2 when compared with wild-type receptor. In contrast, binding of IL-13 to the 4-amino acid mutant form of IL13Rα2 was nearly abolished (Fig. 6B). These data indicate that the Tyr<sup>207</sup>, Asp<sup>271</sup>, Tyr<sup>315</sup>, and Asp<sup>318</sup> residues are not crucial for the interaction of IL13Rα2 (clone 47) mAb with IL13Rα2 but are necessary for binding to IL-13.

N-Linked Glycosylation Affects the Affinity of the IL13Rα2 mAb for IL13Rα2—N-Linked glycosylation has previously been demonstrated to be important for efficient binding of IL-13 to the cognate receptor, IL13Rα2 (30). Taking into consideration the significant overlap in epitope recognition between the IL13Rα2 (clone 47) mAb and IL-13, we hypothesized that N-linked glycosylation of the IL13Rα2 might also contribute to binding of the IL13Rα2 (clone 47) mAb. To test this hypothesis, rhIL13Rα2hFc was treated with Pngase F to remove N-linked glycosylation from the protein. The binding of the IL13Rα2 (clone 47) mAb to control and deglycosylated target protein was investigated. Treatment of rhIL13Rα2 with Pngase F was performed under native conditions (in the absence of SDS) to avoid denaturation of the rhIL13Rα2 affecting the binding of antibodies. Additional mAbs to IL13Rα2 (clones 83807, B-D13, and YY23Z) and rhIL-13 were included in the assay to demonstrate the specificity of binding. In a plate-bound ELISA, binding of the IL13Rα2 (clone 47) mAb to Pngase F-treated IL13Rα2 was decreased by 35% when compared with untreated protein (n = 4; p < 0.001). The binding of the IL13Rα2 (clone 83807) was reduced by 80% when compared with untreated protein and completely absent for the IL13Rα2 mAbs B-D13 and YY-23Z, respectively (n = 4; p < 0.001) (Fig. 7A). Binding of rhIL-13 with Pngase F-treated rhIL13Rα2 was also significantly diminished in agreement with previously published reports (30). To verify that Pngase F treatment resulted in deglycosylation of the protein, control and Pngase F-treated rhIL13Rα2hFc protein was resolved by Western blot. Fig. 7B shows that Pngase F-treated protein has a lower molecular weight, confirming the removal of N-linked glycans from the IL13Rα2 molecule. Binding of the IL13Rα2 (clone 47) mAb to Pngase F-treated U251 glioma and HEK 293 cells expressing wild-type IL13Rα2 was also decreased by ~30% (n = 3; p < 0.05) when compared with control untreated cells (Fig. 7C).

Immunohistochemistry—We evaluated the ability of the IL13Rα2 (clone 47) mAb to detect IL13Rα2 in fresh frozen tissues. Flash frozen human GBM samples or the U251 glioma flank xenograft was stained with either isotype control mlgG1 or the IL13Rα2 (clone 47) mAb. Fig. 8 shows positive (brown) staining in the two human GBM samples albeit with different frequency of positive cells in the sample as well as a U251 glioma cell-based glioma xenograft. Positive staining was detected in two of the three GBM samples analyzed, which is consistent...
FIGURE 6. The contribution of Tyr207, Asp271, Tyr315, and Asp318 residues of IL13Ra2 to the binding of the IL13Ra2 (clone 47) mAb. A, variants of cDNA encoding individual mutations to Ala or a combinatorial 4-amino acid mutant (4aa mut) of IL13Ra2 was generated. HEK cells were transfected with a control vector or a vector encoding the IL13Ra2 variants. After 48 h, binding of the IL13Ra2 (clone 47) mAb to the surface of transfected cells was analyzed by flow cytometry. Anti-IL13Ra2 antibody clones 83807 and B-D13 were used as reference antibodies in this assay. Binding of antibodies was determined as the percentage of positive cells. The ratio of bound clones was determined for each IL13Ra2 mutant and compared with that of the wild-type receptor. One-way analysis of variance followed by Dunnett’s post hoc test was performed. Data represent a summary of four independent experiments. Error bars represent S.D.

B, representative flow histograms demonstrating the binding of clone 47 or rhIL-13 to the WT and 4-amino acid-mutated variant of the IL13Ra2 receptor expressed on the surface of HEK cells. A, area; APC-A, allophycocyanin area.

FIGURE 7. Effect of N-linked glycosylation on the binding of IL13Ra2 to recombinant IL13Ra2. A, binding of IL13Ra2 to control and Pngase F-treated rhIL13Ra2. Plates were coated with rhIL13Ra2 at 1 μg/ml and treated with native buffer or with 1 milliunit/well Pngase F in native buffer for 3 h at 37 °C. An ELISA for binding of the IL13Ra2 (clone 47) mAb in comparison with antibody clones B-D13, 83807, and YY-23Z and rhIL-13 was performed, and the data of one representative experiment from three independent experiments are shown. A paired t test was used to evaluate the difference between control and Pngase F-treated groups (n = 4). *, p < 0.5; **, p < 0.01; ***, p < 0.001. B, a Western blot shows the lower molecular weight of Pngase F-treated rhIL13Ra2 due to removal of N-linked glycosylation adducts from the molecule. C, flow cytometry shows the binding of IL13Ra2 mAbs to IL13Ra2-expressing U251 and HEK293 cells treated with 1 milliunit of Pngase F for 1 h at 37 °C. The data are representative of three independent experiments. A paired t test was used to evaluate the difference between control and Pngase F-treated groups. *, p < 0.5. MFI, mean fluorescence intensity. Error bars represent S.D.
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FIGURE 8. The IL13Rα2 (clone 47) mAb recognizes IL13Rα2 in GBM tissues and in a human glioma xenograft. Immunohistochemistry on frozen tissue sections from three human GBM samples and a U251 xenograft was performed with the IL13Rα2 (clone 47) mAb or mIgG at a concentration of 3 μg/ml. Staining of GBM tissues demonstrates positive staining of the majority of cells in sample 1, positive reactivity in only a fraction of the cells in sample 2, and negative staining in sample 3. Staining in all three samples was performed in the same experiment. Positive staining was also detected in U251 xenograft tissue. Arrows point to individual positive cells. Scale bars = 100 μm.

with the literature suggesting that fewer than 50% of primary GBM express IL13Rα2 (3). These data are also consistent with the ability of this antibody to recognize the native form of IL13Rα2 expressed on the cell surface and in ELISA applications as well as the compromised ability of this mAb to detect denatured antigen by Western blotting.

The Novel IL13Rα2 mAb Prolongs the Survival of Animals with an Intracranial Glioma Xenograft—We next sought to determine the potential therapeutic properties of the IL13Rα2 (clone 47) mAb in an orthotopic mouse model of human glioma. U251 glioma cells were intracranially injected into the brain of nude mice alone, in the presence of control mIgG, or with the IL13Rα2 (clone 47) mAb. Fig. 9A shows that animals in the control PBS (n = 15) and mIgG (n = 16) groups demonstrated a similar median survival of 27 and 25 days, respectively. In contrast, the survival of animals co-injected with the IL13Rα2 (clone 47) mAb (n = 13) was significantly increased to a median of 34 days (p = 0.0001; mIgG versus the IL13Rα2 mAb group). Analysis of H&E staining of the glioma xenografts from brains collected on day 17 revealed a similar pattern of glioma cell distribution in the brain of control groups. In contrast, the tumor mass in the group of animals co-injected with IL13Rα2 mAb was significantly decreased in size (Fig. 9B). Independently, U251 cells were inoculated in the brain of mice and 3 days later injected through the same burr hole with either PBS or the IL13Rα2 (clone 47 or B-D13) mAb as described previously (29). Interestingly, the mice injected with clone 47 demonstrated improvement in median survival when compared with PBS and clone B-D13 groups (35 days versus 27 and 23 days, respectively; n = 7; p > 0.05) (supplemental Fig. 2), similar to what was found in the co-injection experiment (Fig. 9A).

Nevertheless, all animals ultimately succumbed to the disease. These data indicate that the IL13Rα2 (clone 47) mAb may possess the ability to promote tumor rejection of IL13Rα2-expressing U251 glioma cells in the mouse brain. This preliminary finding provides support for further investigation of this antibody for therapeutic purposes in various models and various experimental settings of IL13Rα2-expressing glioma and other malignancies.

DISCUSSION

Recent work has demonstrated that monoclonal antibodies have emerged as valuable research and diagnostic tools as well as therapeutic agents. Monoclonal antibodies specific for tumor-associated antigens have significant advantages over systemic chemotherapies due to the ability to specifically target cancer cells while avoiding interaction with untransformed tissue. Therefore, the search for novel “magic bullets” continues to grow, confirmed by a global market for therapeutic antibodies worth $48 billion as of 2010. Therapeutic antibodies are products of traditional hybridoma technology or screening of libraries for antibody fragments and their subsequent engineering into humanized fragments or full size molecules. Prior to this study, the hybridoma cell line secreting a high affinity antibody to the tumor-specific antigen IL13Rα2 was unavailable to the scientific community. Here, we describe the generation and characterization of a novel high affinity antibody to the tumor-specific antigen IL13Rα2 and discuss its potential use in different applications.

The specificity of interaction of newly discovered antibodies to human IL13Rα2 was analyzed by ELISA using the rhIL13Rα2hFc fusion protein, recombinant human IL13Rα2 expressed on the surface of CHO and HEK cells, and several glioma cell lines expressing IL13Rα2 at various levels by flow cytometry. Our novel antibody demonstrated a specificity of
interaction to human IL13Ra2 and did not cross-react with human IL13Ra1 or mouse IL13Ra2. Moreover, the specificity of binding to IL13Ra2 was confirmed in competitive binding assays using rhIL13Ra2hFc fusion protein by ELISA or by flow cytometry for detection of IL13Ra2 expressed on the surface of HEK cells. In these assays, IL13Ra2 (clone 47) mAb competed with recombinant human IL-13 for its epitope and was able to block ~80% of the binding between IL-13 and IL13Ra2. Conversely, human recombinant IL-13 was able to block ~50% of antibody binding to IL13Ra2. Similarly, a significant decrease in the binding of IL13Ra2 (clone 47) mAb to N10 glioma cells was observed when rhIL13Ra2hFc chimera and rhIL-13 were used as competitors. The binding of rhIL-13 to N10 cells was also abolished by IL13Ra2 (clone 47) mAb. These data indicate that the two molecules have significant overlap in their recognition sites for IL13Ra2.

IL-13 is a small 10-kDa molecule (31), whereas an antibody is ~15 times greater in molecular mass. The ability of rhIL-13 to compete with an antibody for a binding site suggests that the inhibitory property of the antibody is likely due to the specific interaction with amino acid residues contributing to the binding of IL-13 to the cognate receptor rather than to steric hindrance, which can also prevent the interaction of IL-13 with its receptor. Previously, Tyr207, Asp271, Tyr315, and Asp318 were identified as critical residues of IL13Ra2 necessary for interaction with IL-13 (28). Indeed, in our assays, the binding of IL-13 to a mutant IL13Ra2 carrying a combination of all 4 amino acid mutations to alanine was significantly abolished when compared with the wild-type receptor in agreement with previously published work (28). However, binding of the IL13Ra2 mAb to either the individual or the 4-amino acid mutant form of IL13Ra2 was not significantly affected. These findings indicate that Tyr207, Asp271, Tyr315, and Asp318 residues are not critical for the recognition of IL13Ra2 by the IL13Ra2 mAb. The human IL13Ra2 and murine IL13Ra2 are structurally conserved and share 59% amino acid identity (32). Moreover, Tyr207, Asp271, Tyr315, and Asp318 residues are conserved in human and murine IL13Ra2. Absence of binding of the IL13Ra2 mAb to murine IL13Ra2hFc fusion further supports the hypothesis that these amino acid residues contribute to the binding of IL-13 to IL13Ra2 and are not critical for the interaction of this antibody with the receptor.

To further characterize the interaction of IL13Ra2 with our novel antibody, the affinity of the IL13Ra2 mAb was measured and compared with the binding properties of two commercially available antibodies using the surface plasmon resonance method. The affinity of the IL13Ra2 mAb was determined to be equal to 1.39 × 10⁻⁹ M, greatly exceeding the affinity of comparable commercially available antibodies by up to 75X. In agreement with the affinity studies, the IL13Ra2 mAb (clone 47) demonstrated superior to two commercial antibodies binding to the IL13Ra2 expressed on the surface of various glioma cells and in ELISA. Although many properties of antibodies, including the affinity and avidity, in vitro stability, rate of clearance and internalization, tumor penetration, and retention, should be considered prior to specific usage, it has been reported that higher affinity antibodies are better for immuno-therapeutic tumor-targeting applications (33). The single chain antibody fragment (scFv) MR1–1 against epidermal growth factor receptor variant III demonstrates about 15X higher affinity than the parental scFvMR1 and also showed on average a 244% higher tumor uptake than that for the scFvMR1 (34). It is likely that the high affinity properties of our IL13Ra2 mAb will be advantageous for applications utilizing antibodies or associated derivatives for targeting tumor cells expressing IL13Ra2.

Previous work has identified the N-linked glycosylation of IL13Ra2 as a necessary requirement for efficient binding to IL-13 (30). Taking into consideration that our IL13Ra2 mAb inhibits ~80% of IL-13 binding to the cognate receptor, IL13Ra2, it is reasonable to suggest that the binding of this antibody with the deglycosylated form of IL13Ra2 could also be affected. The IL13Ra2 molecule has four potential sites of N-linked glycosylation. The binding of the antibody to rhIL13Ra2 or to IL13Ra2 expressed on the surface of HEK or U251 cells treated with Pnagase F was decreased by 35 and 30%, respectively, when compared with non-treated control. A partial change in binding activity for the clone 47 when compared with clones 83807 and B-D13 suggests that removal of carbohydrate adducts from IL13Ra2 with Pnagase F causes conformational changes of the receptor, indirectly affecting the binding of both IL-13 (30) and the IL13Ra2 mAb to IL13Ra2. This also supports the hypothesis that the antibody binds directly to the IL13Ra2 amino acid backbone rather than due to interaction with post-translational carbohydrate moieties. Supporting this hypothesis, several studies have previously demonstrated that the conformational profile and structural rigidity of proteins depends on N-linked glycosylation (22, 35–38).

To identify whether the IL13Ra2 mAb possesses possible therapeutic properties, we performed an in vivo study whereby glioma cells and the IL13Ra2 (clone 47) mAb were intracranially co-injected into brain, or antibody was injected into established tumor-bearing mice. Interestingly, the IL13Ra2 mAb was able to delay tumor progression and improve survival of animals with intracranial U251 glioma xenografts most significantly in the co-injected model, demonstrating a trend in the improvement of median survival in animals with established glioma. However, further optimization will be necessary to validate the therapeutic effect of this antibody in various experimental settings. Although the underlying mechanism for this antitumor effect remains unclear, the result suggests a possible therapeutic applicability for this antibody alone or as a therapeutic carrier with regard to the future treatment of IL13Ra2-expressing glial and other lineage tumors. Several antibodies have been shown to mediate a cytotoxic effect in tumors through Fc-mediated activation of complement (39). Antibody-dependent cell-mediated cytotoxicity-induced activation of effector cells can also contribute to the cytotoxic effect of antibodies against targeted cells (40, 41). Anti-IL13Ra2 derived from the sera of animals challenged with D5 melanoma cells expressing human IL13Ra2 demonstrates the ability to inhibit cellular growth in vitro (4). However, further detailed studies are required to delineate the properties of this antibody in vivo using additional models of IL13Ra2-expressing tumors.

The IL13Ra2 has been found to be expressed in several types of human cancers, including glioblastoma; medulloblastoma;
Kaposi sarcoma; and head and neck, ovarian, pancreatic, and kidney cancers (2, 43–46). Although the role of IL13Rα2 in some cancers is not yet defined, recent reports have demonstrated that IL13Rα2 contributes to the invasive phenotype of ovarian and pancreatic cancers (5, 13). Moreover, Minn et al. (42) have suggested a relationship between IL13Rα2 expression and breast cancer metastasis to the lung. These studies confirm the importance of further evaluation of the therapeutic properties of clone 47 in additional experimental models of IL13Rα2-overexpressing tumors. Additionally, Fichtner-Feigl et al. (11) demonstrated that the interaction of IL-13 with IL13Rα2 up-regulates TGF-β1, mediating fibrosis in a bleomycin-induced model of lung fibrosis. In light of this finding, it would be interesting to determine whether our novel antibody also has the ability to attenuate TGF-β1-induced pulmonary fibrosis. However, the absence of cross-reactivity with mouse IL13Rα2 will hamper the study of this effect in mouse models, although it may be useful to evaluate other anti-mouse IL13Rα2 antibodies with inhibitory properties, translating those findings into an understanding of the potential implications of clone 47.

In conclusion, we report the generation of a novel antibody specific to human IL13Rα2. The antibody possesses a high affinity for IL13Rα2 and competes with IL-13 for the binding site on IL13Rα2. The antibody recognizes epitopes expressed on the cell surface of glioma cells as well as other IL13Rα2-expressing cells, indicating the potential suitability for targeting IL13Rα2-expressing tumor cells in vivo. These properties combined with the existence of our hybridoma cell line also warrants further engineering of this antibody into smaller antibody fragments for genetic fusion with therapeutic proteins. Moreover, the novel antibody should be tested in various applications, including diagnostic imaging, delivery of antibody radio-nuclide conjugates, bioassays for the detection of IL13Rα2, and as a carrier for therapeutic agents in various types of IL13Rα2-overexpressing tumors. Finally, we have confirmed here that our antibody has applicability in flow cytometry, ELISA, and immunocytochemistry, applications that are critical to study the biological regulation of IL13Rα2 in various contexts.

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