Generation and Characterization of a Single Gene-encoded Single-Chain-Tetravalent Antitumor Antibody

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Abstract

Monoclonal antibody (mAb) CC49, a murine IgG1, reacts with the tumor-associated glycoprotein-72 expressed on a variety of carcinomas. In clinical trials, radiolabeled CC49 has shown excellent tumor localization to a variety of carcinomas. To minimize the immunogenicity of CC49 mAb in patients, a humanized CC49 (HuCC49) was generated by complementarity-determining region (CDR) grafting. The relative affinity of HuCC49 was 2-3-fold less than that of the murine mAb. With the aim of improving tumor targeting, attempts have been made to enhance the avidity of the HuCC49 mAb. Previous research has yielded a single gene-encoded immunoglobulin, SCIgcCC49ACH1, which is a dimer of a single chain consisting of CC49 single-chain Fv linked to the NH2 terminus of the human γ1 Fc through the hinge region. This molecule is comparable to the mouse-human chimeric CC49 in terms of in vitro antigen binding properties, cytolytic activity, and rate of plasma clearance in athymic mice bearing human tumor xenografts. Recently, a dimer of a single-chain consisting of CC49 sFv linked to human 31/Fc via the hinge region was constructed. The diabody, a bivalent antigen-binding structure, is made up of variable heavy (VH)/variable light (VL) domains and VH/VL domains. In each of the variable domain pairs, the VH and VL domains are linked through a short linker peptide. Meanwhile, the two pairs are linked via a 30-residue Gly-Ser linker peptide to yield two antigen-binding sites by lateral and noncovalent association of the VH of one pair with the VL of the other. Transfectomas expressing the single-gene immunoglobulin secrete a homodimer of about Mr 160,000 that reacts to tumor-associated glycoprotein-72. This tetravalent humanized antitumor immunoglobulin molecule may potentially be an efficacious therapeutic and diagnostic reagent against a wide range of human carcinomas.

Introduction

mAb4 CC49, a murine IgG1 (1), reacts with the carcinoma antigen TAG-72 expressed on a variety of carcinomas including adenocarcinomas of the gastrointestinal tract, ovary, breast, endometrium, pancreas, and lung (2). This high-affinity antitumor antibody efficiently targets human colon carcinoma xenografts in nude mice (3). In clinical trials, mAb CC49 has shown excellent tumor localization of carcinomas of the gastrointestinal tract, ovary, and prostate.125I-labeled CC49 has detected 90% of tumors in patients with primary and metastatic colorectal cancer (4). However, as expected of a murine mAb, CC49 elicits HAMA responses in patients (5, 6). HAMA responses render repeated mAb administration ineffective due to an increased rate of antibody clearance from the serum and may also cause allergic reactions in patients. cCC49 mAb was developed to minimize the HAMA response in patients. Later, a HuCC49 mAb was generated by grafting the CDRs of mAb CC49 onto VH and VL frameworks of the human mAbs LEN and 21/28’CL (7). However, the relative affinity of HuCC49 was 2-3-fold lower than that of the murine or cCC49 mAbs.

In an earlier development, a sFv fragment was derived from the murine mAb CC49 and showed rapid plasma and whole body clearance rates in mice and rhesus monkeys (8). The monomeric CC49 sFv and Fab’ have an approximately 8-fold lower equilibrium association constant than that of the dimeric forms of the molecule. Later, a single gene-encoded SCIg (SCIgCC49ACH1; Ref. 9) was developed, which is a homodimer of a single-chain protein consisting of the CC49 sFv linked to the NH2 terminus of the human Fcy1 region through the hinge region. This molecule is comparable to cCC49 in terms of in vitro antigen-binding properties, cytolytic activity, and rate of plasma clearance in athymic mice bearing human tumor xenografts.

To enhance the clinical utility of HuCC49, it is desirable to increase its antigen binding affinity. Because the intact CC49 immunoglobulin, a bivalent molecule, has an 8-fold higher relative binding affinity than the monovalent CC49 sFv (8), one way of enhancing the antigen binding activity of HuCC49 could be by increasing its valency for the antigen. Dimeric antibody fragments or diabodies were designed by generating a pair of antigen-binding sites from two polypeptide chains comprising

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4 The abbreviations used are: mAb, monoclonal antibody; TAG, tumor-associated glycoprotein; CDR, complementarity-determining region; sFv, single-chain Fv; VH, variable heavy; VL, variable light; HAMA, human antimurine antibody; cCC49, mouse-human chimeric CC49; HuCC49, humanized CC49; SCIg, single-chain immunoglobulin; CHOdhfr, dihydrofolate reductase-deficient Chinese hamster ovary; CMV, cytomegalovirus; BSM, bovine submaxillar mucin.

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each polypeptide through a linker that was 5 amino acid residues long, making it feasible to develop a tetravalent HuCC49.

The combination of the variable domains of the other chain by linking the domains within each polypeptide through a linker that was 5 amino acids in length. Direct evidence of the structure of the diabody was later provided by crystallographic studies (11). Combining the diabody approach with the technique of generating SCIgs would make it feasible to develop a tetravalent HuCC49.

In this report, we describe the development of a single gene-encoded SCIg designated SCIgHuCC49db, consisting of a HuCC49 diabody with the COOH terminus of one VL domain linked to the NH2 terminus of the human y1 Fc via the hinge region (Fig. 1). This bivalent antigen-binding structure is made up of a set of VL/VH domains linked to a combination of VH/VL domains. A short Gly-Ser peptide linker of 5 amino acid residues was used to link the heavy and light variable domains within each set, whereas the two sets of domains were linked via a Gly-Ser linker peptide that was 30 residues long. This arrangement allows for the formation of two antigen-binding sites by lateral association of the VL of one pair with the VH of the other. Transfectomas expressing the single-gene expression construct secreted a homodimer with tetrameric binding sites for TAG-72. The relative binding affinity of SCIgHuCC49db was approximately 20-fold higher than that of HuCC49.

### Materials and Methods

**mAbs and Cell Lines.** CHOdhfr- cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM/F12 (Life Technologies, Inc., Grand Island, NY) supplemented with 0.1 mM hypoxanthine, 0.01 mM thymidine, and 10% fetal bovine serum. The medium was also supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. The generation and properties of murine CC49 (1), HuCC49 (7), and SCIgCcCC49ΔCH1 (9) mAbs have been described previously. The horseradish peroxidase-conjugated goat antimouse IgG (Fcγ fragment-specific) and purified polyclonal human IgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

### Synthetic Oligodeoxyribonucleotide Primers for DNA Amplification

The reverse phase cartridge-purified oligonucleotide primers were supplied by Gene Probe (Gaithersburg, MD). Primer sequences are as follows: (a) primer 1 (5' VL1, coding), 5'-GTCGAATTCATTCCATGGATAGCCAGGCCTCGTCG-3'; (b) primer 2 (3' VH1, noncoding), 5'-GCTGCCACCTCACTCCACCTTTCAGTTCACCGCTT-3'; (c) primer 3 (5' VH2, coding), 5'-GGTGAGAGGGGCAGGCTGACGTCG-3'; (d) primer 4 (3' VH2, noncoding), 5'-CTCCACCCCGAGCTCCCGCCCTCCGGCAGAGCCAAGGCTCCGCTCGGCCTCCTCCGAGCCACGCGCTGCCTCGGCCTCCCGCTCCCGCTCCCAGCTGGTGAGGAGGCTCGGGTGGAGGAGGTAGCGGCGGTGGTGGATCTCAGGTCCAGCTGGTGG-3'; (e) primer 5 (5' VH1, coding), 5'-CCAGGAATTCGGTTGGAAGGATCTGTCG-3'.

The overlapping complementary sequences are underlined. The (Gly4-Ser) linker is encoded by the overlap of primers 2 and 3, as well as primers 6 and 7. The long peptide linker (Gly4-Ser)n is encoded by primers 4 and 5. The EcoRI recognition sequence is italicized, whereas the stop codons are in lowercase letters. In the text, the oligonucleotide primers will henceforth be referred to by their designated numbers.

**Templates for DNA Amplification.** To develop a single gene encoding SCIgHuCC49db, sequences encoding the VL1 and VH1 domains were generated by amplifying the VH1 domains from the construct pBacHuCC49sFv, a baculovirus expression construct of HuCC49 sFv. The VL1 sequence was amplified along with its leader sequence. A previously made construct, pdhfrSCIgHuCC49ACH1, was used as a template to generate the humanized VL2 and VH2 sequences by PCR amplification. The VL2 sequence was amplified along with the human y1 hinge and Fc sequences located contiguously 3' to the humanized VH sequence.

**PCR.** The PCR amplification of DNA sequences was carried out by a procedure that has essentially been described previously (9), except for some changes in the parameters of the reaction. Briefly, a 50-μl reaction mixture containing 200 μM of each deoxynucleotide triphosphate, 0.2 μM of each primer, 1 unit of Vent polymerase (New England Biolabs, Beverly, MA), and 50 ng of template DNA was subjected to a 30-s denaturation step at 95°C, followed by 30 cycles of denaturation (30 s at 95°C), annealing (40 s at 55°C), and extension (1 min at 72°C). This was followed by a final primer extension for 15 min at 72°C. Each of the amplified products was purified with the Wizard PCR prep DNA purification system (Promega, Madison, WI).

**Expression Vector and Transfection.** The expression vector pDHFR, which is derived from the plasmid pRC/CMV (Invitrogen, Carlsbad, CA), has been described previously (12). It harbors an enhancer-promoter complex of the immediate early genes of the human CMV for the expression of the target gene.

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Fig. 1  Schematic diagram of the single gene-encoded SCIgHuCC49db mAb. VL and VH, variable region light- and heavy-chain domains; CH2 and CH3, heavy-chain constant region domains of human IgG1.
The plasmid also carries a dhfr expression unit driven by an enhancer-deficient SV40 early promoter and a neomycin resistance gene. CHOdhfr<sup>-</sup> cells, which were seeded at a density of 1 × 10<sup>6</sup> cells/60-mm Petri dish the night before transfection, were transfected with 10 μg of DNA and 30 μl of N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammoniummethyl sulfate cationic liposome reagent (Boehringer Mannheim, Indianapolis, IN) in 150 μl of HEPES-buffered saline, according to the supplier’s instructions. After culturing the cells at 37°C with 5% CO<sub>2</sub> for 16–18 h, the medium was replaced, and the cells were cultured for another 48 h. Subsequently, the cells were seeded into flat-bottomed 96-well plates at a density of 2.4 × 10<sup>5</sup> cells/well in αMEM containing 10% dialyzed fetal bovine serum, 20 mM HEPES, 550 μg/ml G418 (Life Technologies, Inc.), and penicillin/streptomycin.

**Protein Purification.** Tissue culture supernatants were loaded onto a protein G-agarose column (Life Technologies, Inc.). The protein was eluted with 0.1 M glycine buffer (pH 2.5) and the pH value of the eluate was immediately adjusted to 7.0 using 1.0 M Tris (pH 8.0). After the fractions were collected, those containing recombinant proteins (as determined by ELISA) were pooled and concentrated using a Centricon 30 microconcentrator (Amicon, Beverly, MA). Protein concentration was determined using Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL). The purity of the eluted protein was determined by analysis on precast 4–20% SDS-polyacrylamide Tris glycine gel (Novex, San Diego, CA) under reducing and nonreducing conditions. Proteins on the gel were visualized with Coomassie Brilliant Blue R-250.

**ELISA Assay.** ELISA assays were carried out as described previously (7). Individual wells of the 96-well polystyrenic microtiter plates were coated overnight at 4°C with 1.0 μg/well of the TAG-72-positive BSM I-S (Sigma, St. Louis, MO) in coating buffer (1% BSA in PBS). The plates were blocked with 5% BSA in PBS for 1 h at 37°C, washed with PBS containing 1% BSA, and then incubated with 50 μl of culture supernatant of the transfectants for 1 h at 37°C. After washing, the plates were incubated with 50 μl of horseradish peroxidase-conjugated goat antihuman IgG (Fcγ fragment-specific; Jackson Immunoresearch Laboratories, Inc.) in 1% BSA, and then incubated with 50 μl of culture supernatant of the transfectants for 1 h at 37°C. After washing, the plates were incubated with 50 μl of ELISA substrate buffer (0.02 M citric acid and 0.05 M Na<sub>2</sub>HPO<sub>4</sub>) containing 0.03% o-phenylenediamine hydrochloride were added per well. The enzyme reaction was stopped by adding 25 μl of 4 M H<sub>2</sub>SO<sub>4</sub>. The absorbance was read at a wavelength of 490 nm.

**Competition Immunoassay.** For competition assay, biotinylated murine mAb CC49 was used as the labeled antibody, and the TAG-72-positive BSM was used as the target antigen. The remainder of the assay was performed as described previously (13). Briefly, 96-well polystyrenic microtiter plates were coated with BSM I-S, blocked with BSA, and washed as described earlier in this study. Twenty-five μl of the serially diluted test samples in PBS with 1% BSA or buffer control were added to individual wells, followed by 25 μl of 1% BSA in PBS containing 100 ng of biotinylated mouse mAb CC49. Plates were incubated overnight at 4°C, wells were washed, and 50 μl of the Vectastain ABC complex (Vector Laboratories, Burlingame, CA) were then added to each well. After 1 h of incubation at 37°C, wells were washed, and the peroxidase activity was assayed as described earlier. The percentage inhibition of labeled mAb CC49 binding to the TAG-72 antigen was calculated as described previously (9).

**Results**

The Design of a Tetrameric Immunoglobulin, SCIGHuCC49db.

The homodimeric immunoglobulin-like molecule with tetravalent antigen-binding sites is presented schematically in Fig. 1. The design of the SCIG consists of two sets of variable domains: (a) V<sub>H</sub>1/V<sub>L</sub>2; and (b) V<sub>H</sub>2/V<sub>L</sub>1. In the V<sub>H</sub>1/V<sub>L</sub>2 set, the COOH terminus of the V<sub>H</sub>1 is covalently linked to the NH<sub>2</sub> terminus of the V<sub>L</sub>2. In the other set, the COOH terminus of the V<sub>H</sub>2 is linked to the NH<sub>2</sub> terminus of the V<sub>L</sub>1. The variable domains within each set are linked together by a 5-residue (Gly-Ser) linker. A 30-residue-long (Gly-Ser) peptide linker tethers the COOH terminus of the V<sub>H</sub>2 to the NH<sub>2</sub> terminus of the V<sub>H</sub>1 domain. The COOH terminus of the V<sub>L</sub>2 is joined to the NH<sub>2</sub> terminus of the CH<sub>2</sub> domain of the human γ1 Fc via the hinge region. Thus, the V<sub>H</sub>1, V<sub>H</sub>2, V<sub>L</sub>1, V<sub>L</sub>2, and human γ1 Fc domains are part of a single peptide chain, and they are placed in the given order from the NH<sub>2</sub> terminus. The linker joining the variable domains within each set is too short to allow quaternary interaction and proper folding of the V<sub>H</sub>1 and the V<sub>L</sub>2 domains of the same set. The V<sub>H</sub>1 and V<sub>H</sub>2 domains are forced to pair with the complementary domains of the other set by lateral and noncovalent association.

In this SCIG, as in SCIGcc49ACH1 (9) and cc49-interleukin 2 (14), the Cys<sup>229</sup> of the genetic hinge, which held the heavy and light chains of native antibody together, was replaced with a Pro residue. Cys<sup>226</sup> and Cys<sup>229</sup>, which provide the disulfide bonds between the two heavy chains, were retained.

**Generation of a Single-Gene Construct Encoding mAb SCIGHuCC49db.** A schematic flow diagram for the generation of the gene encoding mAb SCIGHuCC49db and its expression construct is shown in Fig. 2. Briefly, sequences encoding V<sub>H</sub>1 and V<sub>L</sub>1, along with its leader, were amplified using the construct pBac9HuCC49sFv as a template, whereas V<sub>H</sub>2 and V<sub>L</sub>2, along with the human Fc sequence, were amplified using the construct pdhfrSCIGHuCC49ACH1 as a template. The latter is a 1089-bp PCR product. Primers 2 and 3 have a 15-bp complementary overlap, which encoded the (Gly<sup>4</sup>-Ser) linker peptide. Primers 4 and 5 carried a 20-bp complementary overlap, and each encoded a part of the (Gly<sup>4</sup>-Ser)<sub>6</sub> linker peptide. Using V<sub>H</sub>1, V<sub>H</sub>2, and V<sub>H</sub>4 amplified DNA fragments as templates and primers 1 and 6 as 5’- and 3’-end primers, a single 1.2-kb DNA fragment encompassing all three sequences was generated. The 3’-end of the 1.2-kb PCR product and the 5’-end of the 1089-bp PCR product have a 15-bp complementing overlap encoding the (Gly<sup>4</sup>-Ser) linker peptide. Using these PCR products as templates and primers 1 and 8 as the 5’- and 3’-end primers, a final 2.3-kb DNA sequence encompassing all three sequences was generated. The 3’-end of the 2.3-kb DNA fragment encompassing all three sequences was generated. The 3’-end of the 1.2-kb PCR product and the 5’-end of the 1089-bp PCR product have a 15-bp complementing overlap encoding the (Gly<sup>4</sup>-Ser) linker peptide. These PCR products as templates and primers 1 and 8 as the 5’- and 3’-end primers, a final 2.3-kb DNA sequence encoding V<sub>H</sub>1, V<sub>H</sub>2, V<sub>H</sub>1, V<sub>L</sub>2, and Fc domains was amplified. The final PCR product, a 2.3-kb DNA fragment, was purified, digested with EcoRI, and inserted into the pCDhfr expression vector at the EcoRI site located downstream from the CMV promoter. The insert in the resulting expression construct, pCDhfr SChuCC49dbFcy1, was sequenced using the Sequenase v.2
schematic representation of the generation of the construct encoding the diabody SCIgHuCC49db. Horizontal numbered arrows, primers for DNA amplification described in “Materials and Methods;” L, leader peptide; LK1, the (Gly4-Ser) linker; LK2, the (Gly4-Ser)3 linker; LK3, the (Gly4-Ser)6 linker; Amp, ampicillin gene; Neo, neomycin gene; CMV, human CMV promoter; dhfr, dihydrofolate reductase gene; BGHpA, bovine growth hormone polyadenylation signal.

**Physical Characterization.** The size and purity of the protein G column-purified antibody was determined by SDS-PAGE. HuCC49 and SCIgcCC49ACH1 were included in the analysis for comparison (Fig. 3). Under nonreducing conditions (Fig. 3A), each of the three molecules showed a single band of apparently high molecular mass (Lanes 1–3). SCIgHuCC49db (Lane 1) showed significantly slower migration than HuCC49 (Lane 2), whereas SCIgcCC49ACH1 (Lane 3) is the fastest-migrating molecule of the three. The single band obtained with SCIgHuCC49db was consistent with a molecular weight of approximately 160,000. Under reducing conditions (Fig. 3B), HuCC49 (Lane 2) shows two distinct bands of 25-27 and 50-55 kDa corresponding to the heavy and light chains of an IgG1 molecule. In contrast, each of the SCIgHuCC49db and SCIgcCC49ACH1 yielded a single band with a molecular mass of approximately 80 and 60 kDa, respectively (Lanes 1 and 3). The single band of SCIgHuCC49db (Lane 1) showed significantly slower migration than the band yielded by mAb SCIgcCC49ACH1 (Lane 3), which, in turn, showed a slower migration than that of the heavy chain of HuCC9 (Lane 2). These results provide convincing evidence that mAb SCIgHuCC49db is expressed as a single-chain protein of approximately 160 kDa and is assembled as a homodimer before secretion.

Fig. 3 SDS-PAGE analysis under nonreducing (A) and reducing (B) conditions. Purified antibodies were electrophoresed on a 4%-20% precast gel. Lanes M, markers (left, size (in kilodaltons)); Lanes 1, SCIgHuCC49db; Lanes 2, HuCC49; Lanes 3, SCIgcCC49ACH1.

system (Amersham Life Science, Arlington Heights, IL), and the construct was used for the transfection of the CHODhfr- cells.

**Development of Transfectomas and Purification of the Antibody.** The expression vector pCdhfr-SCIgHuCC49db was introduced into the CHODhfr- cells by lipofection. Two weeks of drug selection yielded 20 (5.2%) G418+-resistant clones from a total of 384 clones. ELISA assay of culture supernatants from the drug-resistant clones showed that 10 of the clones were positive for binding to TAG-72. The clone that secreted the highest titer of the TAG-72-reactive immunoglobulin was adapted for growth in serum-free medium (CHO-S-SFMII; Life Technologies, Inc.). The antibody was purified from the serum-free culture supernatant by protein G affinity chromatography. The yield was about 1 µg antibody/ml culture supernatant.
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Relative Antigen Binding Affinity. Competition assays were performed to determine the relative binding affinity of SCigHuCC49db, SCIgcCC49ACH1, HuCC49, and the murine mAb CC49 to TAG-72 antigen. Unlabeled immunoglobulins were used to compete with biotinylated murine mAb CC49 for binding to the TAG-72-positive BSM. As shown in Fig. 4, all four mAbs competed completely, and the competition profiles of all four competitors showed similar slopes, indicating that these mAbs bind to the same TAG-72 epitope. Approximately 0.6 nanomole of SCigHuCC49db was required for 50% inhibition of the binding of biotinylated murine CC49 to TAG-72. In contrast, 4.3-, 5.8-, and 20-fold more of SCIgcCC49ACH1, murine CC49, and the CC49 mAbs, respectively, were required to achieve a similar level of inhibition. The negative control antibody, human IgG1, did not compete with the biotinylated murine CC49. These results demonstrate the higher relative binding affinity of SCigHuCC49db compared with other immunoglobulin forms of CC49.

Discussion

For an antibody to be an effective therapeutic reagent against human cancers, it must have minimal immunogenicity to allow repeated antibody administration, optimal antibody affinity and rate of clearance for efficient tumor targeting, and the ability to recruit human effector cells for tumor cytosis.

To minimize immunogenicity of mAb CC49 in patients, a HuCC49 mAb was developed by grafting the CDRs of mAb CC49 onto V\textsubscript{1} and V\textsubscript{\lambda} frameworks of mAbs LEN and 21/28’CL (7). The relative affinities of CC49 and HuCC49 were 2–3-fold less than that of murine CC49. To enhance the clinical potential of HuCC49, it was deemed desirable to increase its functional antigen binding affinity. It has been suggested that the functional affinity of an antibody depends on its valency for its ligand (15, 16). Enhancement of the functional affinity of bivalent antibodies has been achieved by multimerization of the antigen-binding site (17–20). Antibodies with multimeric binding sites have been developed by chemical cross-linking of antibody fragments or by genetically engineering “miniantibodies” and diabodies (for a review, see Ref. 21). To develop a molecule with higher valency, a tetravalent HuCC49 has now been generated. The design of this molecule is based on the structure of a SCig, SCIgcCC49ACH1, that has been reported previously (9).

Unlike the homodimeric SCIgcCC49ACH1, whose single-chain protein consists of CC49 sFv linked to human \gamma1 Fc, the newly generated SCigHuCC49db is a homodimer of a single-chain protein consisting of a HuCC49 diabody (10) genetically tethered to the human \gamma1 Fc. Because the diabody is a bivalent molecule, the homodimeric SCigHuCC49db is tetravalent for its ligand, TAG-72. The HuCC49 diabody is made up of two sets of variable domains, V\textsubscript{1}/V\textsubscript{\lambda} and V\textsubscript{\lambda}/V\textsubscript{1}, linked together via a 30-amino acid peptide linker, whereas a 5-residue linker tethers the two variable domains to each other within each set. Because the linker within each set is too short to allow pairing of variable domains within the set, the variable domain of one set associates laterally and noncovalently with the complementary variable domains of the other set to generate two antigen-binding sites. The SDS-PAGE profile of SCigHuCC49db presented in Fig. 3 provides convincing evidence that the tetravalent antibody is a homodimeric molecule. Under nonreducing conditions, SDS-PAGE analysis of this molecule showed a single band of approximately 160 kDa. Under reducing conditions, a distinct single band of approximately 80 kDa was detected. These data provide support for the schematic model presented in Fig. 1.

It has been shown that blocking of the NH\textsubscript{2} terminus of the V\textsubscript{\lambda} or V\textsubscript{\lambda} domain could adversely affect the antigen binding property of some antibodies (22, 23). In a single-chain diabody construct, the blocking of the NH\textsubscript{2} termini of all variable domains except the first one is inevitable. Therefore, in designing a single-chain diabody, the effect of blocking the NH\textsubscript{2} termini on the ligand binding property is of critical consideration. For mAb CC49, it has previously been demonstrated that the blocking of the NH\textsubscript{2} terminus of either V\textsubscript{\lambda} (9) or V\textsubscript{\lambda}\textsuperscript{5} has no effect on its antigen binding property. Results of the competition assay (Fig. 4) show that mAb SCigHuCC49db has a higher functional affinity for TAG-72 than murine CC49, SCIgcCC49ACH1, or HuCC49 mAbs. Compared with HuCC49, the functional binding affinity of SCigHuCC49db is approximately 20-fold higher. This significantly higher avidity of SCigHuCC49db is consistent with the fact that mAb CC49 recognizes at least two epitopes of TAG-72 (24); the spatial configuration of the four binding sites of SCigHuCC49db in a computer-generated model (data not shown) suggests that all four combining sites of this molecule could simultaneously bind to a multiepitopic antigen.

The single-chain mAb SCigHuCC49db carries a human \gamma1 Fc region. Hence, it is likely to be competent in cytolytic functions, such as antibody-dependent T-cell-mediated cytotoxicity and complement-dependent cytotoxicity. A similarly designed single-chain antibody, SCIgcCC49ACH1, was shown to mediate antibody-dependent T-cell-mediated cytotoxicity against carcinoma cell lines expressing TAG-72 on their cell surface. Exposure of human effector cells to interleukin 2 augmented the antibody-mediated lysis of the target cells (9).
The immunogenicity of SCigHuCC49db has yet to be evaluated. Because it is a humanized antibody, it is likely to be less immunogenic than its murine and chimeric counterparts. However, the possibility that it may elicit an anti-idiotypic response in patients cannot be ruled out at the present time.

These studies demonstrate that, for some antibodies, an enhancement of functional affinity could be achieved by multimerization of the antigen-binding site via diabody formation. Because single-chain SCigHuCC49db has an enhanced functional affinity and is likely to carry out Fc effector functions, it may be an efficacious therapeutic and diagnostic reagent against a wide range of human carcinomas.

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References


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