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Humanization of an anti-p185^{HER2} antibody for human cancer therapy

(antibody engineering/site-directed mutagenesis/c-*erbB-2*/*neu*)

PAUL CARTER*, LEN PRESTA*, CORNELIA M. GORMAN†, JOHN B. B. RIDGWAY†, DENNIS HENNER†, WAI LEE T. WONG‡, ANN M. ROWLAND‡, CLAIRE KOTTS‡, MONIQUE E. CARVER‡, AND H. MICHAEL SHEPARD§

Departments of *Protein Engineering, †Cell Genetics, ‡Medicinal and Analytical Chemistry, and §Cell Biology, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080

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ABSTRACT **The murine monoclonal antibody mumAb4D5, directed against human epidermal growth factor receptor 2 (p185^{HER2}), specifically inhibits proliferation of human tumor cells overexpressing p185^{HER2}. However, the efficacy of mumAb4D5 in human cancer therapy is likely to be limited by a human anti-mouse antibody response and lack of effector functions. A "humanized" antibody, humAb4D5-1, containing only the antigen binding loops from mumAb4D5 and human variable region framework residues plus IgG1 constant domains was constructed. Light- and heavy-chain variable regions were simultaneously humanized in one step by "gene conversion mutagenesis" using 311-mer and 361-mer preassembled oligonucleotides, respectively. The humAb4D5-1 variant does not block the proliferation of human breast carcinoma SK-BR-3 cells, which overexpress p185^{HER2}, despite tight antigen binding (K_d = 25 nM). One of seven additional humanized variants designed by molecular modeling (humAb4D5-8) binds the p185^{HER2} antigen 250-fold and 3-fold more tightly than humAb4D5-1 and mumAb4D5, respectively. In addition, humAb4D5-8 has potency comparable to the murine antibody in blocking SK-BR-3 cell proliferation. Furthermore, humAb4D5-8 is much more efficient in supporting antibody-dependent cellular cytotoxicity against SK-BR-3 cells than mumAb4D5, but it does not efficiently kill WI-38 cells, which express p185^{HER2} at lower levels.**

The protooncogene *HER2* encodes a protein tyrosine kinase (p185^{HER2}) that is homologous to the human epidermal growth factor receptor (1–3). Amplification and/or overexpression of *HER2* is associated with multiple human malignancies and appears to be integrally involved in progression of 25–30% of human breast and ovarian cancers (4, 5). Furthermore, the extent of amplification is inversely correlated with the observed median patient survival time (5). The murine monoclonal antibody mumAb4D5 (6), directed against the extracellular domain (ECD) of p185^{HER2}, specifically inhibits the growth of tumor cell lines overexpressing p185^{HER2} in monolayer culture or in soft agar (7, 8). mumAb4D5 also has the potential of enhancing tumor cell sensitivity to tumor necrosis factor (7, 9). Thus, mumAb4D5 has potential for clinical intervention in carcinomas involving the overexpression of p185^{HER2}.

A major limitation in the clinical use of rodent mAbs is an anti-globulin response during therapy (10, 11). A partial solution to this problem is to construct chimeric antibodies by coupling the rodent antigen-binding variable (V) domains to human constant (C) domains (12–14). The isotype of the human C domains may be varied to tailor the chimeric antibody for participation in antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) (15). Such chimeric antibody molecules are still ≈30% rodent in sequence and are capable of eliciting a significant anti-globulin response.

Winter and coworkers (16–18) pioneered the "humanization" of antibody V domains by transplanting the complementarity determining regions (CDRs), which are the hypervariable loops involved in antigen binding, from rodent antibodies into human V domains. The validity of this approach is supported by the clinical efficacy of a humanized antibody specific for the CAMPATH-1 antigen with two non-Hodgkin lymphoma patients, one of whom had previously developed an anti-globulin response to the parental rat antibody (17, 19). In some cases, transplanting hypervariable loops from rodent antibodies into human frameworks is sufficient to transfer high antigen binding affinity (16, 18), whereas in other cases it has been necessary to also replace one (17) or several (20) framework region (FR) residues. For a given antibody, a small number of FR residues are anticipated to be important for antigen binding. First, there are a few FR residues that directly contact antigen in crystal structures of antibody–antigen complexes (21). Second, a number of FR residues have been proposed (22–24) as critically affecting the conformation of particular CDRs and thus their contribution to antigen binding.

Here we report the rapid and simultaneous humanization of heavy-chain (V_H) and light-chain (V_L) V region genes of mumAb4D5 by using a "gene conversion mutagenesis" strategy (43). Eight humanized variants (humAb4D5) were constructed to probe the importance of several FR residues identified by our molecular modeling or previously by others (22–24). Efficient transient expression of humanized variants in nonmyeloma cells allowed us to rapidly investigate the relationship between binding affinity for p185^{HER2} ECD and antiproliferative activity against p185^{HER2} overexpressing carcinoma cells.

MATERIALS AND METHODS

Cloning of V Region Genes. The mumAb4D5 V_H and V_L genes were isolated by PCR amplification of mRNA from the corresponding hybridoma (6) as described (25). N-terminal sequencing of mumAb4D5 V_L and V_H was used to design the sense-strand PCR primers, whereas the anti-sense PCR primers were based on consensus sequences of murine FR resi-

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Abbreviations: mumAb4D5 and humAb4D5, murine and humanized versions of the monoclonal antibody 4D5, respectively; ECD, extracellular domain; ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; CDR, complementarity-determining region; FR, framework region; V_H and V_L, variable heavy and light domains, respectively; C region, constant region; V region, variable region.

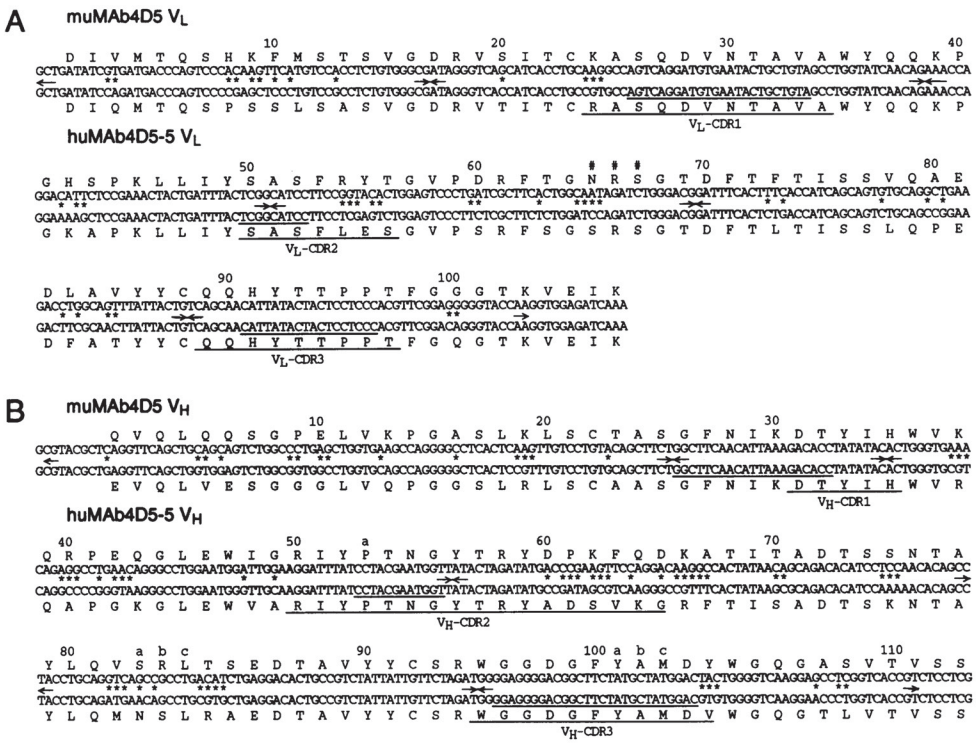

FIG. 1. Nucleotide and amino acid sequences of mumAb4D5 and humAb4D5-5 V_L (*A*) and V_H (*B*) (numbered according to ref. 26). The CDR residues according to a sequence definition (26) and a structural definition (22) are underlined and overlined, respectively. The 5′ and 3′ ends of the oligonucleotides used for gene conversion mutagenesis are shown by arrows and mismatches between genes are shown by asterisks. The asparagine-linked glycosylation site (#) in mumAb4D5 V_L is used in some mumAb4D5 molecules derived from the corresponding hybridoma. However, mumAb4D5 variants, which are glycosylated or aglycosylated in V_L, are indistinguishable in their binding affinity for the $p185^{HER2}$ ECD and in their antiproliferative activity with SK-BR-3 cells (C.K., M. Spellman, and B. Hutchins, unpublished data).

dues (25, 26) incorporating restriction sites for directional cloning shown by underlining and listed after the sequences: V_L sense, 5′-TCCGATATCCAGCTGACCCAGTCTCCA-3′ *Eco*RV; V_L antisense, 5′-GTTTGATCTCCAGCTTGGTACCHSCDCCGAA-3′ *Asp*718; V_H sense, 5′-AGGTSMARCTGCAGSAGTCWGG-3′ *Pst* I; V_H antisense, 5′-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3′ *Bst*EII; where H is A, C, or T; S is C or G; D is A, G, or T; M is A or C; R is A or G; W is A or T. The PCR products were cloned into pUC119 (27) and five clones for each V domain were sequenced by the dideoxynucleotide chain-termination method (28).

Molecular Modeling. Models of mumAb4D5 V_H and V_L domains were constructed by using seven Fab crystal structures from the Brookhaven Protein Data Bank (entries 2FB4, 2RHE, 2MCP, 3FAB, 1FBJ, 2HFL, and 1REI) (29). V_H and V_L of each structure were superimposed on 2FB4 by using main-chain atom coordinates (INSIGHT program, Biosym Technologies, San Diego). The distances from each 2FB4 Cα to the analogous Cα in each of the superimposed structures was calculated. For residues with all Cα–Cα distances $\leq$1Å, the average coordinates for individual N, Cα, C, O, and Cβ atoms were calculated and then corrected for resultant deviations from standard bond geometry by 50 cycles of energy minimization (DISCOVER program, Biosym Technologies) using the AMBER forcefield (30) and fixed Cα atoms. Side chains of FR residues were then incorporated, followed by inclusion of five of the six CDR loops (except V_H–CDR3) using tabulations of CDR conformations (23) as a guide. Side-chain conformations were chosen on the basis of Fab crystal structures, rotamer libraries (31), and packing considerations. Three possible conformations of V_H–CDR3 were taken from a search of similar sized loops in the Brookhaven Protein Data Bank or were modeled by using packing and solvent exposure considerations. Models were then subjected to 5000 cycles of energy minimization.

A model of the humAb4D5 was generated by using consensus sequences derived from the most abundant human subclasses—namely, V_L κ subgroup I and V_H subgroup III (26). The six CDRs were transferred from the mumAb4D5 model onto a human Fab model. All humAb4D5 variants contain human replacements of mumAb4D5 residues at three positions within CDRs as defined by sequence variability (26) but not as defined by structural variability (22): V_L–CDR1 K24R, V_L–CDR2 R54L and V_L–CDR2 T56S.¶ Differences between mumAb4D5 and the human consensus FR residues (Fig. 1) were individually modeled to investigate their possible influence on CDR conformation and/or binding to $p185^{HER2}$ ECD.

Construction of Chimeric Genes. Genes encoding the chimeric mAb4D5 light and heavy chains were separately assembled in previously described phagemid vectors containing the human cytomegalovirus enhancer and promoter, a 5′ intron, and simian virus 40 polyadenylylation signal (32). Briefly, gene segments encoding mumAb4D5 V_L (Fig. 1*A*) and REI human κ_1 light-chain C_L (33) were precisely joined as were genes for mumAb4D5 V_H (Fig. 1*B*) and human IgG1 C region (34) by subcloning (35) and site-directed mutagenesis as described (36). The IgG1 isotype was chosen, as it is the preferred human isotype for supporting ADCC and CDC by using matched sets of chimeric (15) or humanized antibodies (17). The PCR-generated V_L and V_H fragments (Fig. 1) were subsequently mutagenized so that they faithfully represent the sequence of mumAb4D5 determined at the protein level: V_H, Q1E; V_L, V104L and T109A. The human IgG1 C regions are identical to those reported (37) except for the mutations E359D and M361L (Eu numbering; ref. 26), which we installed to convert the antibody from the naturally rare A allotype to the much more common non-A allotype (26). This was an attempt to reduce the risk of anti-allotype antibodies interfering with therapy.

Construction of Humanized Genes. Genes encoding chimeric mAb4D5 light-chain and heavy-chain Fd fragment (V_H and C_H1 domains) were subcloned together into pUC119 (27) to create pAK1 and were simultaneously humanized in a single step (43). Briefly, sets of six contiguous oligonucleotides were designed to humanize V_H and V_L (Fig. 1). These oligonucleotides are 28–83 nucleotides long, contain 0–19 mismatches to the murine antibody template, and are con-

¶Variants are denoted by the amino acid residue and number followed by the replacement amino acid.

strained to have 8 or 9 perfectly matched residues at each end to promote efficient annealing and ligation of adjacent oligonucleotides. The sets of V_H and V_L humanization oligonucleotides (5 pmol each) were phosphorylated with either ATP or [γ-^{32}P]ATP (36) and separately annealed with 3.7 pmol of pAK1 template in 40 μl of 10 mM Tris·HCl (pH 8.0) and 10 mM $MgCl_2$ by cooling from 100°C to ≈20°C over ≈20 min. The annealed oligonucleotides were joined by incubation with T4 DNA ligase (12 units; New England Biolabs) in the presence of 2 μl of 5 mM ATP and 2 μl of 0.1 M dithiothreitol for 10 min at 14°C. After electrophoresis on a 6% acrylamide sequencing gel, the assembled oligonucleotides were located by autoradiography and recovered by electroelution. The assembled oligonucleotides (≈0.3 pmol each) were simultaneously annealed to 0.15 pmol of single-stranded deoxyuridine-containing pAK1 prepared as described (38) in 10 μl of 40 mM Tris·HCl (pH 7.5) and 16 mM $MgCl_2$ as described above. Heteroduplex DNA was constructed by extending the primers with T7 DNA polymerase and transformed into *Escherichia coli* BMH 71-18 *mutL* as described (36). The resultant phagemid DNA pool was enriched first for human V_L by restriction purification using *Xho* I and then for human V_H by restriction selection using *Stu* I as described (36, 39). Resultant clones containing both human V_L and human V_H genes were identified by nucleotide sequencing (28) and designated pAK2. Additional humanized variants were generated by site-directed mutagenesis (36). The mumAb4D5 V_L and V_H gene segments in the transient expression vectors described above were then precisely replaced with their humanized versions.

Expression and Purification of mAb4D5 Variants. Appropriate mAb4D5 light- and heavy-chain cDNA expression vectors were cotransfected into adenovirus-transformed human embryonic kidney cell line 293 by a high-efficiency procedure (32). Media were harvested daily for up to 5 days and the cells were refed with serum-free medium. Antibodies were recovered from the media and affinity purified on protein A-Sepharose CL-4B (Pharmacia) as described by the manufacturer. The eluted antibody was buffer-exchanged into phosphate-buffered saline by G25 gel filtration, concentrated by ultrafiltration (Amicon), sterile-filtered, and stored at 4°C. The concentration of antibody was determined by both total IgG and antigen binding ELISAs. The standard used was humAb4D5-5, whose concentration had been determined by amino acid composition analysis.

Cell Proliferation Assay. The effect of mAb4D5 variants on proliferation of the human mammary adenocarcinoma cell line SK-BR-3 was investigated as described (6) by using saturating mAb4D5 concentrations.

Affinity Measurements. mAb4D5 variant antibodies and p185^{HER2} ECD were prepared as described (40) and incubated in solution until equilibrium was found to be reached. The concentration of free antibody was then determined by ELISA using immobilized p185^{HER2} ECD and was used to calculate affinity (K_d) as described (41). The solution-phase equilibrium between p185^{HER2} ECD and mAb4D5 variants was found not to be grossly perturbed during the immobilized ECD ELISA measurement of free antibody.

RESULTS

Humanization of mumAb4D5. The mumAb4D5 V_L and V_H gene segments were first cloned by PCR and sequenced (Fig. 1). The V region genes were then simultaneously humanized by gene conversion mutagenesis using preassembled oligonucleotides. A 311-mer oligonucleotide containing 39 mismatches to the template directed 24 simultaneous amino acid changes required to humanize mumAb4D5 V_L. Humanization of mumAb4D5 V_H required 32 amino acid changes, which were installed with a 361-mer containing 59 mismatches to the mumAb4D5 template. Two of eight clones sequenced precisely encode humAb4D5-5, although one of these clones contained a single nucleotide imperfection. The six other clones were essentially humanized but contained a small number of errors: <3 nucleotide changes and <1 single nucleotide deletion per kilobase. Additional humanized variants (Table 1) were constructed by site-directed mutagenesis of humAb4D5-5.

Expression levels of humAb4D5 variants were 7–15 μg/ml as judged by ELISA using immobilized p185^{HER2} ECD. Successive harvests of five 10-cm plates allowed 200–500 μg of each variant to be produced in a week. Antibodies affinity purified on protein A gave a single band on a Coomassie blue-stained SDS/polyacrylamide gel of mobility consistent with the expected mass of ≈150 kDa. Electrophoresis under reducing conditions gave two bands consistent with the expected mass of free heavy (48 kDa) and light (23 kDa) chains (data not shown). N-terminal sequence analysis (10 cycles) gave the mixed sequence expected (see Fig. 1) from an equimolar combination of light and heavy chains.

humAb4D5 Variants. In general, FR residues were chosen from consensus human sequences (26) and CDR residues were chosen from mumAb4D5. Additional variants were constructed by replacing selected human residues in humAb4D5-1 with their mumAb4D5 counterparts. These are V_H residues 71, 73, 78, 93, plus 102 and V_L residues 55 plus 66. V_H residue 71 has previously been proposed by others (24) to be critical to the conformation of V_H–CDR2. Amino acid sequence differences between humAb4D5 variant molecules are shown in Table 1 together with their p185^{HER2} ECD

Table 1. p185^{HER2} ECD binding affinity and anti-proliferative activities of mAb4D5 variants

mAb4D5 variant	V_H residue 71 (FR3)	73 (FR3)	78 (FR3)	93 (FR3)	102 (CDR3)	V_L residue 55 (CDR2)	66 (FR3)	K_d, nM	Relative cell proliferation
humAb4D5-1	R	D	L	A	V	E	G	25	102
humAb4D5-2	Ala	D	L	A	V	E	G	4.7	101
humAb4D5-3	Ala	Thr	Ala	Ser	V	E	G	4.4	66
humAb4D5-4	Ala	Thr	L	Ser	V	E	Arg	0.82	56
humAb4D5-5	Ala	Thr	Ala	Ser	V	E	Arg	1.1	48
humAb4D5-6	Ala	Thr	Ala	Ser	V	Tyr	Arg	0.22	51
humAb4D5-7	Ala	Thr	Ala	Ser	Tyr	E	Arg	0.62	53
humAb4D5-8	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.10	54
humAb4D5	Ala	Thr	Ala	Ser	Tyr	Tyr	Arg	0.30	37

Human and murine residues are shown in one-letter and three-letter amino acid codes, respectively. K_d values for the p185^{HER2} ECD were determined by the method of Friguet *et al.* (41) and the standard error of each estimate is ±10%. Proliferation of SK-BR-3 cells incubated for 96 hr with mAb4D5 variants is shown as a percentage of the untreated control as described (7). Data represent the maximal antiproliferative effect for each variant (see Fig. 2) calculated as the mean of triplicate determinations at a mAb4D5 concentration of 8 μg/ml. Data are all taken from the same experiment and the estimated standard error is ±15%.

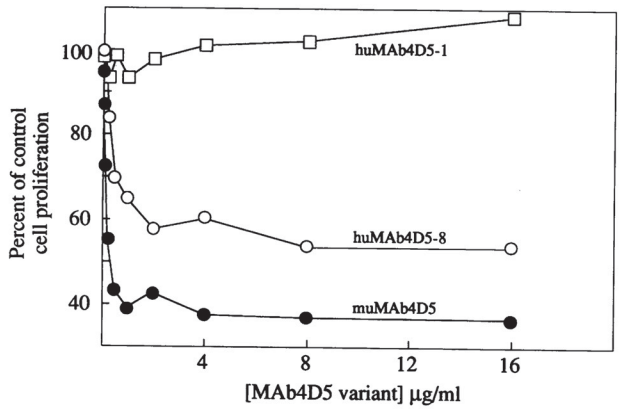

FIG. 2. Inhibition of SK-BR-3 proliferation by mAb4D5 variants. Relative cell proliferation was determined as described (7) and data (average of triplicate determinations) are presented as a percentage of results with untreated cultures for mumAb4D5, humAb4D5-8, and humAb4D5-1.

binding affinity and maximal antiproliferative activities against SK-BR-3 cells. Very similar K_d values were obtained for binding mAb4D5 variants to either SK-BR-3 cells (C.K. and N. Dua, unpublished data) or to p185^{HER2} ECD (Table 1).

The most potent humanized variant designed by molecular modeling, humAb4D5-8, contains five FR residues from mumAb4D5. This antibody binds the p185^{HER2} ECD 3-fold more tightly than does mumAb4D5 itself (Table 1) and has comparable antiproliferative activity with SK-BR-3 cells (Fig. 2). In contrast, humAb4D5-1 is the most humanized but least potent mumAb4D5 variant, created by simply installing the mumAb4D5 CDRs into the consensus human sequences. humAb4D5-1 binds the p185^{HER2} ECD 80-fold less tightly than does the murine antibody and has no detectable antiproliferative activity at the highest antibody concentration investigated (16 μg/ml).

The antiproliferative activity of humAb4D5 variants against p185^{HER2} overexpressing SK-BR-3 cells is not simply correlated with their binding affinity for the p185^{HER2} ECD—e.g., installation of three murine residues into the V_H domain of humAb4D5-2 (D73T, L78A, and A93S) to create humAb4D5-3 does not change the antigen binding affinity but does confer significant antiproliferative activity (Table 1).

The importance of V_H residue 71 (24) is supported by the observed 5-fold increase in affinity for p185^{HER2} ECD on replacement of R71 in humAb4D5-1 with the corresponding murine residue, A71 (humAb4D5-2). In contrast, replacing V_H L78 in humAb4D5-4 with the murine residue A78 (humAb4D5-5) does not significantly change the affinity for the p185^{HER2} ECD or change antiproliferative activity, suggesting that residue 78 is not of critical functional significance to humAb4D5 in interacting with p185^{HER2} ECD.

V_L residue 66 is usually a glycine in human and murine κ-chain sequences (26) but an arginine occupies this position in the mumAb4D5 κ light chain. The side chain of residue 66 is likely to affect the conformation of V_L–CDR1 and V_L–CDR2 and the hairpin turn at residues 68–69 (Fig. 3). Consistent with the importance of this residue, the mutation V_L G66R (humAb4D5-3 → humAb4D5-5) increases the affinity for the p185^{HER2} ECD by 4-fold with a concomitant increase in antiproliferative activity.

From molecular modeling, it appears that the side chain of mumAb4D5 V_L Y55 may either stabilize the conformation of V_H–CDR3 or provide an interaction at the V_L–V_H interface. The latter function may be dependent on the presence of V_H Y102. In the context of humAb4D5-5 the mutations V_L E55Y (humAb4D5-6) and V_H V102Y (humAb4D5-7) individually increase the affinity for p185^{HER2} ECD by 5-fold and 2-fold, respectively, whereas together (humAb4D5-8) they increase the affinity by 11-fold. This is consistent with either proposed role of V_L Y55 and V_H Y102.

Secondary Immune Function of humAb4D5-8. humAb4D5-8 efficiently mediates ADCC against SK-BR-3 breast carcinoma cells, which overexpress p185^{HER2} at high levels as anticipated from its IgG1 isotype (Table 2). In contrast, humAb4D5-8 is very inefficient in mediating ADCC against the normal lung epithelium cell line WI-38, which expresses p185^{HER2} at 100-fold lower levels than SK-BR-3 cells (Table 2). The murine parent antibody is not very effective in mediating ADCC against either SK-BR-3 or WI-38 cells.

DISCUSSION

mumAb4D5 is potentially useful for human therapy since it is cytostatic toward human breast and ovarian tumor lines overexpressing p185^{HER2}. Here we have humanized mumAb4D5 in an attempt to improve its potential clinical efficacy by reducing its immunogenicity and tailoring the Fc region to support ADCC and possibly CDC.

Rapid humanization of humAb4D5 was facilitated by the gene conversion mutagenesis strategy developed here using long preassembled oligonucleotides. This method uses less

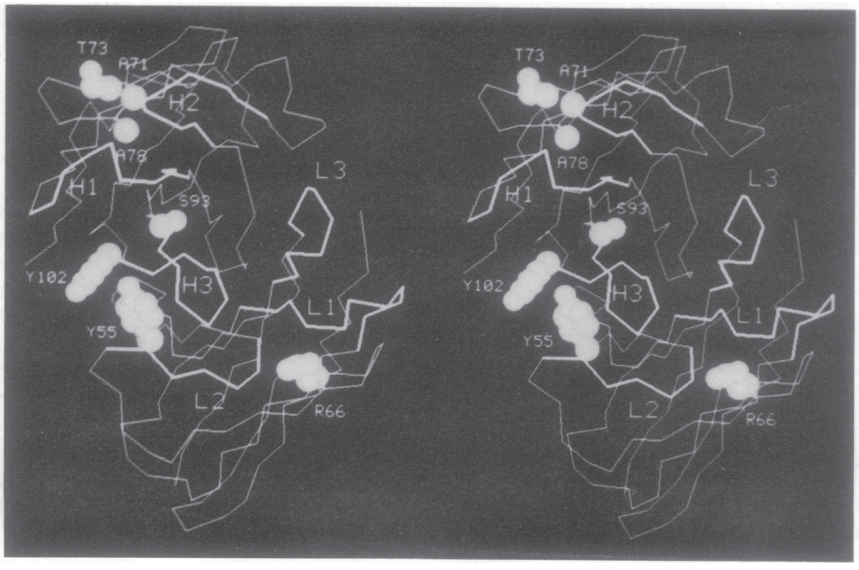

FIG. 3. Stereoview of α-carbon tracing for model of humAb4D5-8 V_L and V_H. The CDR residues (26) are shown in boldface and side chains of V_H residues A71, T73, A78, S93, and Y102 and V_L residues Y55 and R66 (see Table 1) are shown.

Table 2. Selectivity of ADCC mediated by mAb4D5 variants

Effector/target ratio	WI-38		SK-BR-3	
	mumAb4D5	humAb4D5-8	mumAb4D5	humAb4D5-8
Antibody concentration, 100 ng/ml				
25:1	<1.0	9.3	7.5	40.6
12.5:1	<1.0	11.1	4.7	36.8
6.25:1	<1.0	8.9	0.9	35.2
3.13:1	<1.0	8.5	4.6	19.6
Antibody concentration, 10 ng/ml				
25:1	<1.0	3.1	6.1	33.4
12.5:1	<1.0	1.7	5.5	26.2
6.25:1	1.3	2.2	2.0	21.0
3.13:1	<1.0	0.8	2.4	13.4

Sensitivity to ADCC of human cell lines WI-38 (normal lung epithelium) and SK-BR-3 (breast tumor), which express 0.6 and 64 pg of $p185^{HER2}$ per μg of cell protein, respectively, as determined by ELISA (40). ADCC assays were carried out as described (15) using interleukin 2 activated human peripheral blood mononuclear cells as effector cells and either WI-38 or SK-BR-3 target cells in 96-well microtiter plates for 4 hr at 37°C at different antibody concentrations. Values given represent percentage specific cell lysis as determined by ^{51}Cr release. The estimated standard error in these quadruplicate determinations was ±10%.

than half the amount of synthetic DNA, as does total gene synthesis, and does not require convenient restriction sites in the target DNA. Our method appears to be simpler and more reliable than a similar protocol recently reported (42). Transient expression of humAb4D5 in human embryonic kidney 293 cells permitted the isolation of 0.2- to 0.5-mg humAb4D5 variants for rapid characterization by growth inhibition and antigen binding affinity assays. Furthermore, different combinations of light and heavy chain were readily tested by cotransfection of corresponding cDNA expression vectors.

The crucial role of molecular modeling in the humanization of mumAb4D5 is illustrated by the designed variant humAb4D5-8, which binds the $p185^{HER2}$ ECD 250-fold more tightly than the simple CDR loop swap variant humAb4D5-1. It has previously been shown that the antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling (17, 20). Here we have designed a humanized antibody that binds its antigen 3-fold more tightly than the parent antibody and is almost as potent in blocking the proliferation of SK-BR-3 cells. While this result is gratifying, assessment of the success of molecular modeling must await the outcome of ongoing x-ray crystallographic structure determination.

humAb4D5-8 also supports cytotoxicity via ADCC against SK-BR-3 tumor cells in the presence of human effector cells but is not effective in directing the killing of normal (WI-38) cells, which express $p185^{HER2}$ at much lower levels. This augurs well for the ongoing treatment of human cancers overexpressing $p185^{HER2}$ by using humAb4D5-8.

We thank Bill Henzel for N-terminal sequence analysis of mAb4D5 variants; Nancy Simpson for sequencing the cDNAs for mumAb4D5 V-region genes; Maria Yang for providing the C_L-containing clone; Susie Wong for performing amino acid composition analysis; Irene Figari for performing the ADCC assays; Mark Vasser, Parkash Jhurani, Peter Ng, and Leonie Meima for synthesizing oligonucleotides; Bob Kelley for helpful discussions; and Tony Kossiakoff for support.

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