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CHIMERIC AND HUMANIZED ANTIBODIES WITH SPECIFICITY FOR THE CD33 ANTIGEN[1]

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L and H chain cDNAs of M195, a murine mAb that binds to the CD33 Ag on normal and leukemic myeloid cells, were cloned. The cDNAs were used in the construction of mouse/human IgG1 and IgG3 chimeric antibodies. In addition, humanized antibodies were constructed which combined the complementarity-determining regions of the M195 antibody with human framework and constant regions. The human framework was chosen to maximize homology with the M195 V domain sequence. Moreover, a computer model of M195 was used to identify several framework amino acids that are likely to interact with the complementarity-determining regions, and these residues were also retained in the humanized antibodies. Unexpectedly, the humanized IgG1 and IgG3 M195 antibodies, which have reshaped V regions, have higher apparent binding affinity for the CD33 Ag than the chimeric or mouse antibodies.

The mAb M195 is a murine IgG2a antibody reactive with the CD33 Ag (1–3). M195 binds to early myeloid cells, some monocytes, and cells of most myeloid leukemias but not to the earliest hematopoietic stem cells. The M195 Ag is also absent from any other hematopoietic or nonhematopoietic tissue. These properties make the antibody an ideal candidate for therapy of AML[3] and chronic myelogenous leukemia. The efficient cellular binding and internalization of M195 have allowed use of the radiolabeled antibody in trials for AML therapy. These trials have demonstrated specific targeting of ^{131}I-M195 to leukemic cells in the bone marrow (4). The murine M195 antibody, however, does not kill leukemic cells by complement-dependent cytotoxicity with human complement or by antibody-dependent cellular cytotoxicity with human effector cells. The HAMA response may also preclude long term use of the murine antibody in patients.

To increase the effector function and reduce the immunogenicity of murine antibodies, rDNA technology can be applied to construct chimeric antibodies (5). Such antibodies combine the V region of a mouse antibody with a human antibody C region, thus retaining the binding specificity of the murine antibody while presenting less foreign amino acid sequence to the human immune system. In some but not all cases, chimeric antibodies in fact have improved complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity function relative to mouse antibodies (6) or are less immunogenic in human patients (7). However, because the entire V domain of a chimeric antibody, about one third of the molecule, is of mouse origin, chimeric antibodies may still provoke a substantial HAMA response when used to treat humans.

To reduce further the immunogenicity of murine antibodies, Winter and colleagues (8–10) constructed reshaped or "humanized" antibodies by combining only the smallest required part of a mouse antibody, the CDR, with human V region frameworks and C regions. A disadvantage of this approach is that the CDR may adopt a new conformation after being grafted onto the human framework so the humanized antibody often has substantially reduced affinity for the Ag (10). To overcome this problem, we have previously chosen a human framework as homologous as possible to the original mouse framework and used computer modeling to identify several key residues in the mouse framework which contact the CDR (11–12). These residues must also be transferred to the human framework to maintain the conformational integrity of the CDR.

In this paper we describe the cloning of the murine M195 H and L chain V region cDNAs by a new method. Chimeric antibodies of the human IgG1 and IgG3 isotypes were constructed to improve the effector functions of the mouse antibody. Applying our general procedures, we then designed and synthesized humanized M195 antibodies that retain the binding site of the murine antibody while having the potential to reduce immunogenicity further.

MATERIALS AND METHODS

Cloning of V region cDNAs. The V domain cDNAs for the H and L chains of murine M195 were cloned by an anchored PCR method (13), which is outlined in Figure 1. First, total RNA was prepared using the hot phenol method. Briefly, 1×10^7 M195 hybridoma cells were resuspended in 1.2 ml of RNA extraction buffer (50 mM sodium acetate, pH 5.2 , 1% SDS), vortexed, and incubated at room temperature for 2 min. The cell lysates were then incubated with 0.6 ml of phenol, pH 5.2, at 65°C for 15 min followed by a 15-min incubation on ice. The extract was spun in a microcentrifuge; the aqueous phase was recovered and ethanol precipitated twice. The RNA pellet

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession numbers M83098 and M83099.
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[3] Abbreviations used in this paper: AML, acute myelogenous leukemia; HAMA, human anti-mouse antibody; PCR, polymerase chain reaction; CDR, complementarity-determining region.

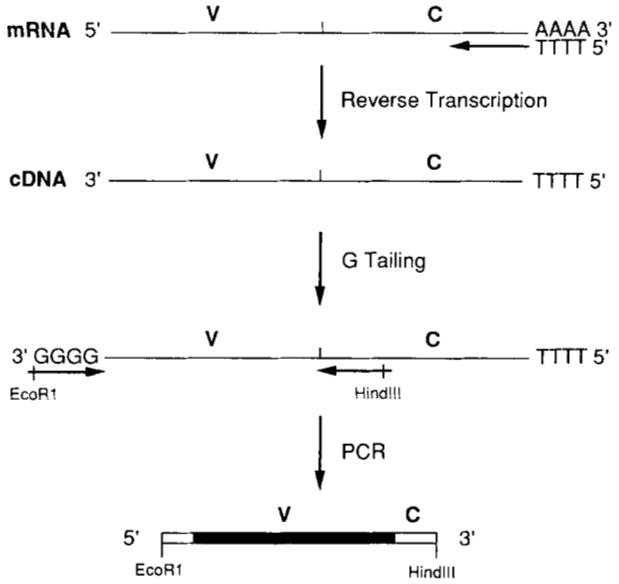

Figure 1. Scheme for cloning of light and H chain V region cDNAs. *Horizontal arrrows* represent oligonucleotide primers.

was resuspended in H_2O and quantitated by an OD_{260} reading. cDNA was synthesized from the RNA by incubating 5 μg of total RNA with 40 ng of dT_{12-18} (Pharmacia LKB Biotechnology Inc., Piscataway, NJ), 200 U of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Bethesda, MD), 40 U of RNasin (Promega Biotec, Madison, WI), 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, and a 0.5 mM concentration of each dNTP in a 20-μl reaction volume for 60 min at 37°C. The cDNA was purified by phenol extraction and ethanol precipitation. A tail of dGs was added to the 3′-terminus of the cDNA by incubating it with 15 U of terminal deoxynucleotidyl transferase (Bethesda Research Laboratories), 0.1 M potassium cacodylate, pH 7.2, 2 mM $CoCl_2$, 0.2 mM dithiothreitol, and 1 mM dGTP in a 20-μl reaction volume for 30 min at 37°C. Under the conditions described, G-tails generally contained about 20 bases. One half of the G-tailed product was then amplified to clone the V_L gene and the other half amplified to clone the V_H gene, using Taq polymerase. The V_L gene was amplified with the primers TATATCTAGAATTCCCCCCCCCCCCCCC CCC and TATAGAGCTCAAGCTTGGATGGTGGGAAGATGGATAC AGTTGGTGC, which, respectively, anneal to the G-tail and the κ L chain C region. The V_H gene was amplified similarly, but using the downstream primer TATAGAGCTCAAGCTTCCAGTGGATAGAC(C AT)GATGGGG(GC)TGT(TC)GTTTTGGC, which anneals to the C region of γ chains. The sequences in parentheses indicate base degeneracies, which were introduced so the primer would be able to recognize all γ chains isotypes. *Eco*RI and *Hin*dIII sites were included in the upstream and downstream primers for convenient subcloning. An alternate set of restriction sites (*Xba*I and *Sac*I) was also included in the primers for the rare event that an *Eco*RI or *Hin*dIII site is present in the V region genes. The PCR reactions were performed in a programmable heating block using 30 rounds of temperature cycling (92°C for 1 min, 50°C for 2 min, and 72°C for 3 min). The reactions included the G-tailed product, 1 μg of each primer, and 2.5 U of Taq polymerase (Perkin-Elmer Cetus Instruments, Norwalk, CT) in a final volume of 100 μl, with the reaction buffer recommended by the manufacturer. The PCR product bands were excised from a low melting agarose gel, digested with restriction enzymes, and cloned into the pUC18 vector for sequence determination.

Construction of expression vectors. pVk (Fig. 2*A*) was constructed by replacing the *Eco*RI-*Xba*I fragment in the pVκ1 plasmid (11) with a 600-bp *Fnu*DII fragment containing a human CMV enhancer and promoter (14), to which appropriate linkers were attached. The same fragment was inserted similarly into the pVγ1 plasmid. A fragment in the modified plasmid containing the *hyg* gene was then replaced with a fragment containing a mutant gene for dihydrofolate reductase (15) to construct pVg1 (Fig. 2*B*). The plasmid pVg3 was constructed similarly to pVg1, with a 3400-bp *Hin*dIII-*Pvu*II fragment containing the genomic $C_{\gamma 3}$ gene (16) replacing the $C_{\gamma 1}$ gene, using linkers so the *Xba*I and *Bam*HI sites were preserved. All reactions were carried out under standard conditions (17).

ends of the M195 V_L cDNA clone. The 5′-primer contained an *Xba*I site followed by the ATG codon and the next 15 nucleotides of V_L. The 3′-primer included the last 21 nucleotides of the V_L coding sequence (noncoding strand) followed by the 17 nucleotides that follow the $J_κ1$ segment in mouse genomic DNA and then an *Xba*I site. The fragment generated by PCR with these primers was digested with *Xba*I and cloned into the *Xba*I site of the pVk vector. The correct orientation and sequence of the cloned V_L segment in pVk were verified by sequencing. The chimeric H chain gene was constructed similarly, using PCR with appropriate primers on the M195 V_H cDNA and cloning into the *Xba*I sites of pVg1 and pVg3.

Construction of humanized genes. Nucleotide sequences were selected which encode the protein sequences of the humanized M195 H and L chains, including signal peptides, generally utilizing codons found in the mouse sequence. Several degenerate codons were changed to create restriction sites or to remove undesirable ones. For each V region gene, two pairs of overlapping oligonucleotides on alternating strands were synthesized (Applied Biosystems 380B DNA synthesizer), which encompassed the entire coding sequences as well as a splice donor signal, and contained suitable restriction sites at their ends. The oligonucleotides were 110 to 140 bases long with 15-base overlaps. dsDNA fragments were synthesized with Klenow polymerase from the 5′-pair and separately from the 3′-pair of oligonucleotides, digested with restriction enzymes, ligated into the pUC18 vector, and sequenced. A 5′-fragment and a 3′-fragment with correct sequences were then excised from pUC18 and ligated together into the *Xba*I sites of the expression vectors pVk, pVg1, or pVg3.

Transfection. Transfection was by electroporation using a Gene Pulser apparatus (Bio-Rad) at 360 V and 25-microfaraday capacitance according to the manufacturer's instructions. Before transfection, the L- and H chain-containing plasmids were linearized using *Bam*HI, extracted with phenol-chloroform, and ethanol precipitated. All transfections were done using 20 μg of each plasmid DNA and about 10^7 Sp2/0 cells (ATCC CRL 1581) in PBS. The cells from each transfection were plated into one 96-well tissue culture plate. After 48 h selective medium (DMEM + 10% FBS + HT media supplement (Sigma) + 1 μg/ml mycophenolic acid) was applied. After the wells had become confluent with surviving colonies of cells, medium from each well was assayed for the presence and quantity of secreted antibodies by ELISA. A high yielding clone from each transfection was grown up to produce antibody for purification.

Purification of antibodies. The chimeric IgG1 and IgG3 and humanized IgG1 antibodies were purified from culture supernatant by affinity chromatography on protein A-Sepharose CL-4B (Pharmacia). The bound antibodies were eluted with 0.1 M glycine HCl (pH 3.0) and neutralized with 1 M Tris-HCl (pH 8.0). The buffer was exchanged into PBS by passing over a PD10 column (Pharmacia) or by dialysis. The humanized IgG3 antibody was purified similarly but using protein G-agarose (Pierce Chemical Co., Rockford, IL) instead of protein A-Sepharose. The isotypes of the purified antibodies were verified using an Ouchterlony immunodiffusion kit (The Binding Site Ltd., Birmingham, UK). The final antibody concentration was determined assuming that 1 mg/ml has an A_{280} of 1.35.

Affinity measurements. The purified antibodies were labeled with $Na^{125}I$ using chloramine-T, to 2 to 10 μCi/μg of protein. Scatchard analysis was made by binding dilutions of labeled antibody to 10^5 HL60 cells for 90 min at 0°C. The cells were washed in RPMI medium and counted. To avoid nonspecific and FcR binding, the assays were done in the presence of human serum. The least squares method was used to fit a line to the plot of bound/free vs bound antibody, and the apparent K_a was read from the slope of the line.

RESULTS

Cloning of V region cDNAs. The M195 H and L chain V domain cDNAs were cloned using an anchored PCR method (Fig. 1), with 3′-primers that hybridized to the C regions and 5′-primers that hybridized to the G-tails. Immunoglobulin chains have been cloned previously by PCR using mixed 5′-primers (18, 19), but that method may not yield the true sequence of the 5′-part of the chain and may not suffice to clone chains with unusual 5′-sequences. Two independent M195 L chain clones obtained by our method were sequenced and found to be identical; similarly, two H chain clones had the same sequence. The nucleotide sequences of the L and H chain V regions have been deposited in GenBank; the translated

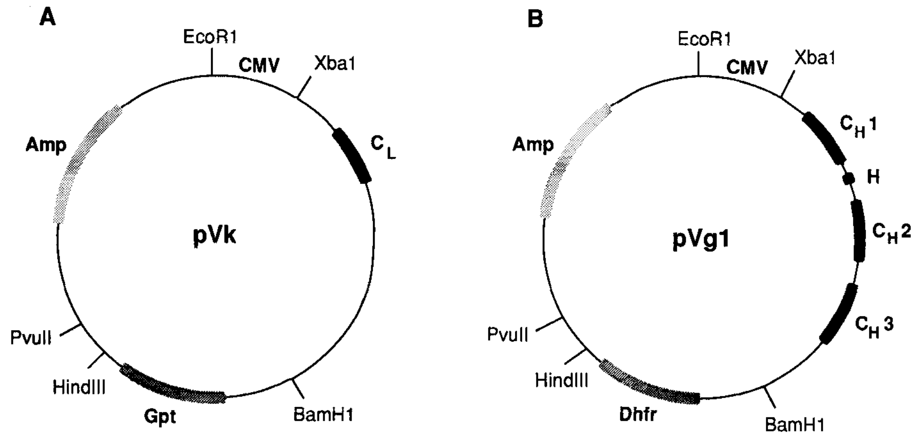

Figure 2. Diagram of plasmid vectors. Coding regions are shown as *boxes*, and important restriction sites are indicated. Not drawn to scale.

Figure 3 (*upper lines*). The V_L domain belongs to the mouse κ chain subgroup III and uses the $J_\kappa1$ segment. The V_H domain belongs to the mouse H chain subgroup II and uses the J_H4 segment (20).

Expression vectors. Plasmid vectors were constructed for the expression of chimeric and humanized L and H chain genes. The plasmid pVk (Fig. 2*A*) contains the human genomic C_κ segment (21) including about 300 bp of the preceding intron and the poly(A) signal. The intron is preceded by a unique *Xba*I site into which a L chain V region can be cloned. Transcription of the complete L chain gene will be initiated by the strong human CMV major immediate early promoter and enhancer (14). The pVk plasmid also contains a selectable marker gene for xanthine-guanine phosphoribosyltransferase (*gpt*) (22). Plasmids pVg1 (Fig. 2*B*) and pVg3 for expression of $\gamma1$ and $\gamma3$ H chain genes are similar to pVk but, respectively, contain the $C_{\gamma1}$ and $C_{\gamma3}$ C region genes (16, 23) and another selectable marker. The H chain C region genes are genomic clones that include the C_H1, hinge, C_H2, and C_H3 exons with the intervening introns and about 200 bp of the intron preceding C_H1. The mutant dihydrofolate reductase selectable marker can be used for gene amplification (24).

Synthesis of chimeric antibodies. PCR with appropriate primers was used to precisely copy the V_L region, including the signal sequence and J segment, from an M195 L chain cDNA clone. The 5′-PCR primer was designed to insert an *Xba*I site before the ATG codon. The 3′-primer was designed to insert the splice donor signal that normally follows mouse $J_\kappa1$ in genomic DNA after the J segment, followed by an *Xba*I site. The PCR- generated fragment was then cloned into the *Xba*I site in the pVk vector (Fig. 2*A*). Doing so created a complete chimeric κ L chain gene with a miniintron between the mouse V-J and human C_κ segments. Similarly, PCR with suitable primers was used to insert the M195 V_H region, including the signal sequence and J segment and followed by a splice donor signal, into the *Xba*I sites of pVg1 (Fig. 2*B*) and pVg3. The resulting plasmids contain complete chimeric $\gamma1$ and $\gamma3$ H chain genes, respectively, with a miniintron between the mouse V-J segment and the human C_H1 exon.

The chimeric L chain-containing plasmid was transfected into Sp2/0 mouse myeloma cells together with either the chimeric $\gamma1$- or chimeric $\gamma3$-containing plasmid. Antibody secreted by the transfected cell clones was determined by ELISA. The best producing clones secreted 2.5 μg of chimeric IgG1 and 6 μg of chimeric IgG3/10^6 cells/24 h. Chimeric IgG1 and IgG3 antibodies secreted into the medium were purified on protein A-Sepharose. Human IgG3 antibodies generally do not bind protein A (25), so the ability of the chimeric IgG3 antibody to bind to protein A may be mediated through the V region (26). The purified IgG1 and IgG3 chimeric antibodies were analyzed by SDS-PAGE (Fig. 4). There is some heterogeneity in the murine and chimeric H chains, which may be a result of variable glycosylation.

Design of humanized M195 V domain. To retain the binding affinity and specificity in the humanized antibodies, the general procedures of Queen et al. (11) were followed. First, a human antibody V region with maximal sequence homology to the mouse M195 V region was selected to provide the framework sequences for the humanized antibodies in order to minimize the chance that the CDR conformation would be distorted when grafted onto the human framework. The L and H chain V regions were taken from the same human antibody to reduce the possibility of incompatibility in assembly of the two chains. Based on a sequence homology search against the National Biomedical Research Foundation Protein Identification Resource, the Eu antibody was selected to provide the framework sequences for humanization of M195. The complete Eu L and H chain V regions are, respectively, 54 and 51% homologous to the murine M195 L and H chain V regions.

Next, the computer programs ABMOD and ENCAD (27) were used to construct a molecular model of the M195 V domain. Inspection of the refined model of murine M195 revealed several amino acid residues in the framework which have significant contacts with the CDR residues. Specifically, residues Phe-40, Ile-52, and Asp-74 in the L chain interact with the CDR. Residues Tyr-27, Thr-30, Ile-48, Lys-67, Ala-68, Arg-98, and Trp-106 in the H chain also show significant interactions with the CDR. These murine residues were retained in the V region framework of the humanized M195 antibodies.

Different human L and H chain V regions exhibit substantial amino acid homology within the framework regions (20). However, a given V region usually contains amino acids atypical of other human V regions at several framework positions. The Eu antibody contains such unusual residues at positions L10, L67, and L110 in the

A

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1    D I V L T Q S P A S L A V S L G Q R A T
     D I Q M T Q S P S S L S A S V G D R V T

21   I S C R A S E S V D N Y G I S F M N W F
     I T C R A S E S V D N Y G I S F M N W F

41   Q Q K P G Q P P K L L I Y A A S N Q G S
     Q Q K P G K A P K L L I Y A A S N Q G S

61   G V P A R F S G S G S G T D F S L N I H
     G V P S R F S G S G S G T D F T L T I S

81   P M E E D D T A M Y F C Q Q S K E V P W
     S L Q P D D F A T Y Y C Q Q S K E V P W

101  T F G G G T K L E I K
     T F G Q G T K V E I K
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B

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1    E V Q L Q Q S G P E L V K P G A S V K I
     Q V Q L V Q S G A E V K K P G S S V K V

21   S C K A S G Y T F T D Y N M H W V K Q S
     S C K A S G Y T F T D Y N M H W V R Q A

41   H G K S L E W I G Y I Y P Y N G G T G Y
     P G Q G L E W I G Y I Y P Y N G G T G Y

61   N Q K F K S K A T L T V D N S S S T A Y
     N Q K F K S K A T I T A D E S T N T A Y

81   M D V R S L T S E D S A V Y Y C A R G R
     M E L S S L R S E D T A V Y Y C A R G R

101  P A M D Y W G Q G T S V T V S S
     P A M D Y W G Q G T L V T V S S
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Figure 3. Sequences of the humanized M195 light (*A*) and heavy (*B*) chain V domains (*lower lines*), aligned with the respective murine M195 V domains (*upper lines*). *Vertical marks* indicate identity of the amino acids. The CDR are *underlined*. Residues in the Eu framework which were replaced by murine residues or consensus human residues are *double underlined*.

consensus human residue rather then the Eu residue in the humanized antibody. Several of the unusual residues in Eu occur in the H chain J segment, which is significantly mutated from any human genomic J_H segment. The final amino acid sequences of the humanized M195 L and H chain V regions are shown in Figure 3, aligned with the murine sequences.

Synthesis of humanized antibodies. DNA segments encoding the humanized M195 L and H chain V regions were constructed by total gene synthesis from overlap-

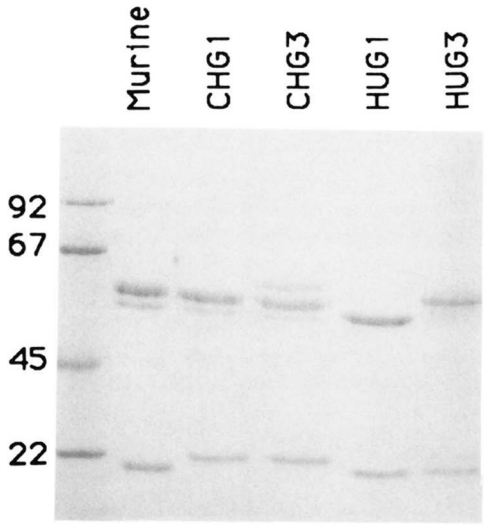

Figure 4. SDS-PAGE analysis of M195 antibodies. 1 μg of each antibody was run on the 10% gel, which was stained with Coomassie blue. *Left lane*, m.w. standards of the indicated sizes. *CHG1*, chimeric antibody of IgG1 isotype; *CHG3*, chimeric antibody of IgG3 isotype; *HUG1*, humanized antibody of IgG1 isotype; *HUG3*, humanized antibody of IgG3 isotype.

TABLE I
Affinities of M195 antibodies

Antibody	Apparent K_a ($\times 10^9$ M^{-1})
Murine	2.21 ± 0.73[a]
Chimeric IgG1	2.28
Chimeric IgG3	0.69 ± 0.13[a]
Humanized IgG1	5.90 ± 1.4[a]
Humanized IgG3	3.33

[a] The average of three independent experiments is shown ± the SD.

were surrounded by *Xba*I sites. The DNA segments were inserted into the *Xba*I sites of the appropriate expression vectors (Fig. 2) to produce plasmids containing complete humanized κ, γ1, and γ3 genes. The humanized L chain-containing plasmid was transfected into Sp2/0 cells together with either the humanized γ1- or humanized γ3-containing plasmid, and cells were selected for expression of the *gpt* gene. The best antibody-producing clones secreted 8 μg of humanized IgG1 and 3 μg of humanized IgG3/10^6 cells/24 h. IgG1 antibody secreted into the medium was purified on protein A-Sepharose. Unlike the chimeric IgG3 antibody, the humanized IgG3 antibody did not bind protein A, so it was purified on protein G-agarose. The humanized IgG1 and IgG3 antibodies were shown to be pure by SDS-PAGE (Fig. 4). The H chain isotypes of the humanized and chimeric antibodies were verified by Ouchterlony immunodiffusion.

Affinity measurements. Flow cytometry was used to show that the chimeric and humanized M195 antibodies bind to HL60 and U937 cells, which express the CD33 Ag, but not to several cell lines that do not express CD33 (data not shown). Binding of the radiolabeled chimeric and humanized antibodies to HL60 cells was specific and saturable. The apparent affinities for the CD33 Ag of murine M195 and the various genetic constructs were determined by Scatchard analysis (Table I). Murine M195 was found to have an apparent K_a of 2.2×10^9 M^{-1} (Fig. 5*A*), consistent with previous results (1). Chimeric IgG1 M195 has the same apparent affinity as murine M195 whereas the chimeric IgG3 has a slightly lower apparent

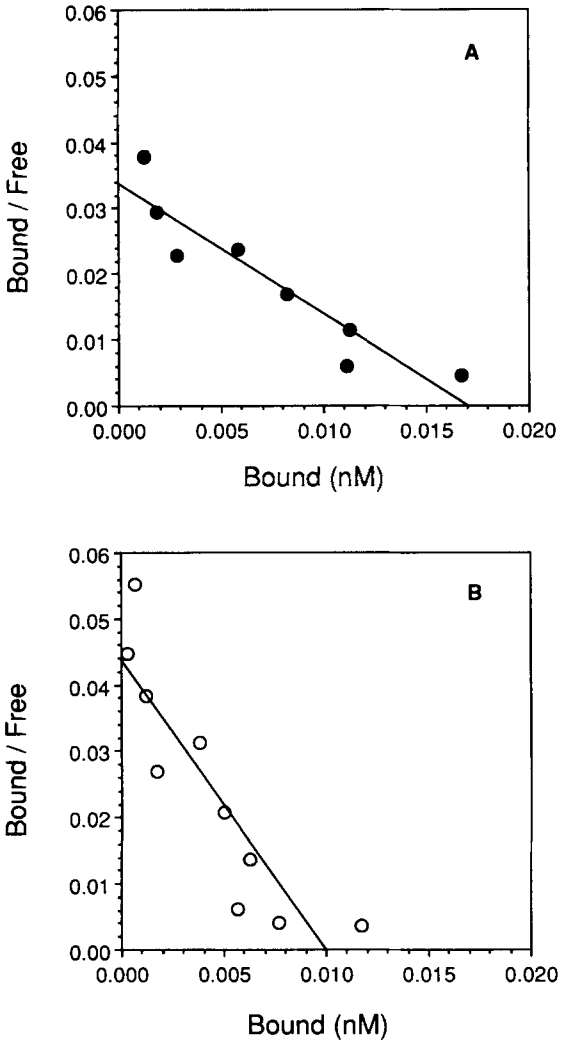

Figure 5. Representative Scatchard analyses of binding of ^{125}I-labeled murine (*A*) and humanized IgG1 (*B*) M195 antibodies to HL60 cells.

3-fold higher than murine M195. The affinity determinations of the murine M195 and humanized IgG1 antibodies were performed three times to verify this finding, each time with very similar results (Table I). The humanized IgG3 antibody has an apparent affinity slightly higher than the murine antibody but not as high as the humanized IgG1 form. Repeating the experiments suggested that the chimeric and humanized IgG3 antibodies were not completely stable in solution over time.

DISCUSSION

To date, a multitude of chimeric antibodies and at least seven fully humanized antibodies have been described. The humanized antibodies, respectively, bind to the pan-lymphocyte CAMPATH-1 Ag (10), the p55 chain of the IL-2 receptor (11), the gB and gD glycoproteins of herpesvirus (12), the fusion protein of respiratory syncytial virus (28), the CD4 Ag (29) and, as described here, the CD33 Ag. Because the V and C regions of antibodies are independent protein domains (20), it is possible to construct a chimeric antibody by replacing the mouse C region with a human C region without altering the conformation of the V region, including the CDR. Hence for the Ag. In contrast, construction of a humanized antibody by replacing the mouse V region framework with a human framework may alter key contacts between the framework and the CDR, which are required to maintain the CDR conformation (30). The altered CDR conformation may in turn reduce or abolish the binding affinity of the antibody for the Ag.

To retain high affinity when humanizing a mouse antibody, we have previously introduced two concepts (11). First, a human framework is selected which maximizes homology with the sequence of the mouse antibody V region. Such a framework is less likely to introduce distortions into the CDR. For the humanized M195 antibody, we chose the framework of the human Eu antibody, which we have also used to humanize the anti-Tac and anti-gD antibodies (11, 12). Eu is used frequently because it is the only human antibody having an H chain in subgroup I for which the sequence of the L chain is also available (20), and the H chains of many murine mAb are most homologous to human subgroup I. However, we have also successfully used the framework of the human Pom antibody and of other human antibodies when they provide greater homology (12) and (M. S. Co, unpublished data). The utility of choosing a homologous human framework has recently been specifically confirmed by other investigators (29).

In addition to selecting an appropriate human framework, we use a computer model of the murine antibody to identify a small number of amino acids in the mouse framework which make key contacts with the CDR. These residues are retained in the humanized antibody. In humanized M195, three L chain and seven H chain murine residues were kept for this reason. It may be argued that the mouse framework amino acids will make the humanized antibody more immunogenic when used in human patients. However, when humanized anti-Tac, which incorporates a number of murine framework residues, was tested in cynomolgus monkeys, any monkey antibody response was directed to the anti-Tac CDR and not to the framework (31). For the fully humanized antibodies that have been examined (CAMPATH-1 (10), anti-respiratory syncytial virus (28), anti-CD4 (29), and anti-Tac (W. Schneider and C. Q., unpublished data)) using a homologous human framework or retaining some murine framework residues or both have been shown to be critical for retaining high binding affinity. For the M195 antibody, these methods actually led to a humanized antibody with higher apparent binding affinity, as determined by Scatchard analysis, than the mouse or chimeric antibodies. Elsewhere, we have also directly verified the increased apparent binding affinity of humanized M195 relative to murine M195 by competition binding of one antibody against the other[4] (36). The reason for the increase in affinity of M195 upon humanization is under investigation but may relate to a change in glycosylation patterns.

Initial animal and clinical studies with chimeric and humanized antibodies have been encouraging. Humanized CAMPATH-1 has induced remissions in two patients with non-Hodgkin lymphoma (32) and one patient with

[4] **Caron, P. C., M. S. Co, M. K. Bull, N. M. Avdalovic, C. Queen, and D. Scheinberg.** 1991. Humanized M195 (anti-CD33) monoclonal antibodies:

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