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# CONSTRUCTION OF A CHIMERIC ANTIBODY WITH THERAPEUTIC POTENTIAL FOR CANCERS WHICH OVEREXPRESS c-erbB-2

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**SUMMARY:** We describe the chimerization of a monoclonal antibody directed against the c-erbB-2 protein using a novel PCR method for cloning immunoglobulin variable region genes. We also describe the characterization of the chimera and show its potential use for treating cancers which overexpress the c-erbB-2 protein. The genomic DNA fragments of heavy and light chain variable genes were cloned by PCR using uniquely designed primers which allowed for isolation of genes containing functional promoters, signal and coding sequences. The chimeric genes were then constructed by linking variable regions of murine genes to human constant $\gamma 1$ and $\kappa$ genes. Expression of the chimeric immunoglobulin genes resulted in production of properly assembled chimeric antibody with improved biological properties. 

The presence of cell surface tumor-associated antigens is a characteristic of many cancers. One such tumor-associated antigen, c-erbB-2, has been shown to be overexpressed in a variety of cancers including gastrointestinal, non-small cell lung, breast, and ovarian adenocarcinomas (1-7). Studies of the c-erbB-2 gene in human breast and ovarian cancers have found that amplification of the gene occured in 25 to 30% of both types of tumors and the amplification is correlated with overexpression of the protein product. The gene amplification and overexpression are associated with poor clinical prognosis and shortened overall patient survival (5, 8, 9). These data strongly suggest that c-erbB-2 plays a role in the pathogenesis of certain types of human cancer.

The use of murine monoclonal antibodies for human therapy is limited by immune responses in humans elicited by the mouse derived antibody molecule (8-12).

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Mouse/human chimeric antibodies have been constructed in an effort to minimize the immunogenicity of the immunoglobulin molecule while maintaining the binding specificity and affinity of the antibody (13-16). In many applications, chimeric antibodies have demonstrated improved effector function in complement-mediated tumor cell lysis and in antibody-dependent cellular cytotoxicity assays as compared to the parental murine monoclonal antibody (17-20). In this work, we describe the construction of the chimeric antibody, BACh 250, in which the heavy and light chain variable regions of an anti-c-erbB-2 mouse monoclonal antibody were cloned using uniquely designed PCR primers allowing for genomic cloning with intact promoter, signal and coding sequences. The engineered chimeric antibody was characterized and the binding specificity and affinity of BACh 250 to c-erbB-2 antigen were determined. Most importantly, the chimeric antibody was shown to have biological properties which make it attractive as a therapeutic for tumors which overexpress the c-erbB-2 protein.

## MATERIALS AND METHODS

*Cells, vectors, probes and primers.* Mouse myelomas P3x63-Ag8.653 (P3X) and SP2/0, and the human cell lines ARH-77 and SKOV-3 were obtained from American Type Culture Collection (ATCC, Rockville, MD). SKBR-3 cells and NIH3T3 transfectants expressing the human c-erbB-2 gene were kindly provided by Dr. S. Aaronson (NIH, Bethesda, MD). The development and characterization of TAb 250 has been previously described (21). Plasmid vectors pSV2neo, pRSVgpt, pBR322 and pUC19 were obtained from ATCC, and pIBI 21 was obtained from IBI (New Haven, CT). The mouse heavy chain $J_H$ probe was a 1.0 Kb DNA fragment containing the $JH_3$ and $JH_4$ regions, which was isolated from plasmid pJ3J4. The mouse light chain $J_\kappa$ probe was a 1.8 Kb DNA fragment containing the $J_\kappa$ region, which was isolated from plasmid pJKHB.1. Both pJ3J4 and pJKHB.1 were generous gifts from Dr. J. Donald Capra (Southern Medical Center, University of Texas). The probes used to identify human constant heavy and light chain genes were oligonucleotides, designed from the coding sequences of both genes. By using the GCG sequence analysis software (22), three degenerative 5' primers and one specific 3' primer were designed for $V_H$ cloning. Six degenerative 5' primers and one specific 3' primer were designed for $V_\kappa$ cloning. For both $V_H$ and $V_\kappa$ amplification, the PCR primers were designed to amplify the variable regions, including their own promoters, leaders and variable region coding sequences. The $V_H$ gene fragment also contains its own enhancer sequence.

*Cloning of chimeric heavy and light chain genes.* Two genomic DNA fragments, one containing TAb 250 heavy chain variable region ($V_H$) and the other containing TAb 250 light chain kappa variable region ($V_\kappa$) were both amplified and cloned from hybridoma genomic DNA by PCR. Southern hybridizations with $J_H$ and $J_\kappa$ probes were performed to verify that the PCR amplified fragments were immunoglobulin heavy and light chain variable regions. The PCR amplified TAb 250 $V_H$ and $V_\kappa$ genes were subsequently cloned into plasmid vector pUC19 and pIBI21 respectively, and their nucleotide sequences were determined by sequencing both strands of the template DNA. The mouse light chain enhancer, which was not included in the cloned $V_\kappa$ gene fragment, was cloned separately from TAb 250 genomic DNA by PCR.

The human heavy chain $\gamma$-1 constant region gene ($C_\gamma$-1) and the human light chain kappa region gene ($C_\kappa$) were cloned from the human IgG producing cell line ARH-77 using a similar PCR approach as described above. The PCR products were

verified by Southern hybridization using oligonucleotide probes to $C_\gamma$-1 and $C_\kappa$ coding regions. The amplified $C_\gamma$-1 and $C_\kappa$ gene fragments contained from sixty to two hundred base pairs of flanking intron sequences on the 5' and 3' ends.

*Gene transfection.* Samples of 5 μg to 50 μg of linearized heavy and light chain plasmid DNA were cotransfected into $1x10^7$ SP 2/0 cells and P3X cells by electroporation. Culture supernatants harvested from G418 resistant transfectants were screened for chimeric heavy chain and chimeric light chain expression by two-antibody sandwich ELISAs.

*Characterization of chimeric antibody by metabolic labeling and immunoprecipitation.* An aliquot of $1x10^7$ SP2/0 cells was metabolically labeled with 300 μCi of Tran$^{35}$S-label (ICN, specific activity = 1100 ci/mmole) and immunoprecipitation carried out essentially as described (23). Culture supernatants or cell lysates were incubated with 10 μg of goat anti-human kappa antibody (Sigma Chemical Co.) followed by 50 μl of protein A-Sepharose CL-4B (Pharmacia). Samples were analyzed on a 4-20% SDS polyacrylamide gradient gel under non-reducing condition or on a 12% SDS polyacrylamide gel under reducing conditions.

*Binding assays.* The binding activity of chimeric antibody, BACh 250, was tested in both ELISA and competitive binding assays. The ELISA was performed in 96-well microtiter plates coated with glutaraldehyde fixed c-erbB-2 transfected NIH 3T3 cells at $1x10^4$ cells per well and goat anti-human IgG (H+L)-horseradish peroxidase conjugate (Zymed Lab Inc.). In the competitive binding assay, BACh 250 was tested for its ability to compete with $^{125}$I-TAb 250 in binding to c-erbB-2 protein on the surface of SKBR-3 cells as described (24).

*Biological characterization.* The ability of BACh 250 to inhibit tumor cell growth *in vitro* was compared to the murine TAb 250 antibody using SKOV-3 cells as described (23). For complement-mediated cytotoxicity, $^{51}$Cr-release assays were carried out using standard procedures (25). Rabbit complement (Cedarlane Labs, Ontario, CAN) was added to $^{51}$Cr-labeled SKBR-3 cells followed by either TAb or BACh 250. The mean cpm of duplicate wells was determined, and percent specific release was calculated using the following formula: (Experimental cpm from test well - spontaneous cpm from complement control wells/total cpm released from detergent wells - spontaneous cpm) x 100. Release from the control wells containing cells alone or cells plus antibody only, varied from 0-3%. The standard deviation of duplicates was less than 10%.

For antibody-dependent cellular cytotoxicity assays, effector cells were obtained by separating human peripheral blood mononuclear cells from the leukophoresed blood of healthy donors (Stanford blood bank) by standard procedures. Effector cells were then resuspended with $^{51}$Cr-labeled SKBR-3 cells and ADCC determined (25) after 24 h. The mean cpm of duplicate wells was determined and percent specific release was calculated using the following formula: (Experimental cpm from test well - spontaneous cpm from target alone wells/total cpm released from detergent wells - spontaneous cpm) x 100. Release from control wells containing target cells plus antibody only did not differ from the release from wells which contained target cells alone. The standard deviation of duplicates was less than 10%.

To examine the effects of BACh250 on *in vivo* tumor growth, SKOV-3 tumor cells were implanted and passaged in 4-6 wk old BAlb/c nu/nu mice as previously described (23).

## RESULTS

*Cloning and construction of chimeric Ab heavy and light chain genes.* Both TAb 250 $V_H$ and $V_\kappa$ genes were cloned from TAb 250 hybridoma genomic DNA by PCR. The amplified DNA fragment obtained from PCR using the heavy chain primers was ~2.0 Kb (Figure 1 ). This 2.0 Kb band was amplified by one of the three designed

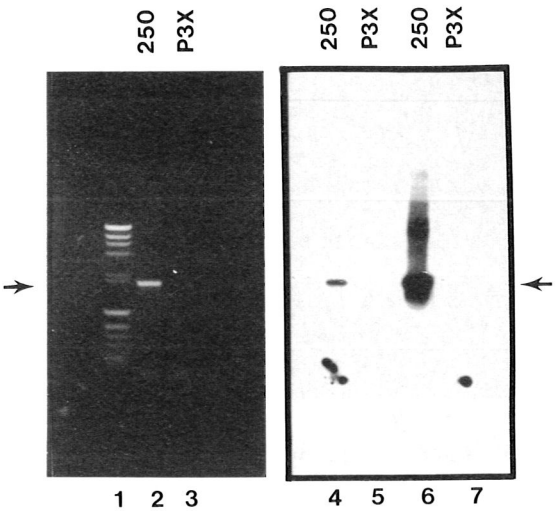


**Figure 1. TAb 250 $V_H$ gene cloned by PCR.** Aliquots of the PCR reaction mix using primers designed to amplify the TAb 250 $V_H$ region were analyzed on a 0.8% agarose gel (lanes 1-3) and by a Southern blot (lanes 4-7). Lane 1, DNA markers (23, 9.4, 6.5, 4.3, 2.3, 2.0, 1.0, 0.77, 0.6, 0.5 and 0.39 Kb); Lane 2, 4 and 6, the amplification from genomic DNA of TAb 250 hybridoma; Lane 3, 5 and 6, the amplification from genomic DNA of P3X cells. The amplified TAb 250 $V_H$ DNA band is indicated by an arrow. The difference between Lane 4, 5 and Lane 6, 7 is the amount of PCR reaction mix loaded.

heavy chain PCR primer sets (5' primer: 5'-GAGGAATTC(AC)TATAG-CAG(GA)A(AC)(GC)A(CT)ATGCAAAT-3'; 3' primer: 5'-GAGGAATTCGTCGACTAAA-TACATTTTAGAAGTCGAT-3'). The amplified DNA fragment obtained from $V_\kappa$ PCR cloning was ~3.0 Kb (Figure 2), and was amplified by one of the six light chain PCR primer sets designed for $V_\kappa$ cloning ( 5' primer: 5'-(AC)A(AT)TTACTTCCTTATTTG-(GA)TGACT(GA)-CTTTGCAT-3'; 3' primer: CATTAAGCTTTTAATATAACACTG-GATA-3'). These two PCR amplified fragments were verified to contain the immunoglobulin variable genes for heavy and light chains respectively by Southern hybridization analyses using probes from $J_H$ and $J_\kappa$ regions (Figures 1 and 2). In addition, both PCR amplified $V_H$ DNA and $V_\kappa$ DNA were amplified from TAb 250 hybridoma DNA, but not from the fusion partner P3X DNA, indicating the cloned $V_H$ and $V_\kappa$ genes were rearranged specific for TAb 250. The nucleotide sequences of the cloned $V_H$ and $V_\kappa$ genes were obtained from sequencing independent clones. The regions of the promoter, leader, coding region (V, D, and J) and enhancer of TAb 250 $V_H$ gene were all identified and mapped as shown in Figure 3A. A similar map for TAb 250 $V_k$ gene is shown in Figure 3B. The light chain enhancer was cloned from TAb 250 genomic DNA in a separate PCR (data not shown). As indicated in Figure 3, the TAb 250 $V_H$ gene rearranged to $J_4$ and $V_\kappa$ gene rearranged to $J_2$. The amino acid sequences of $V_H$ and $V_\kappa$ proteins obtained from direct $NH_2$-terminal amino acid sequencing of purified

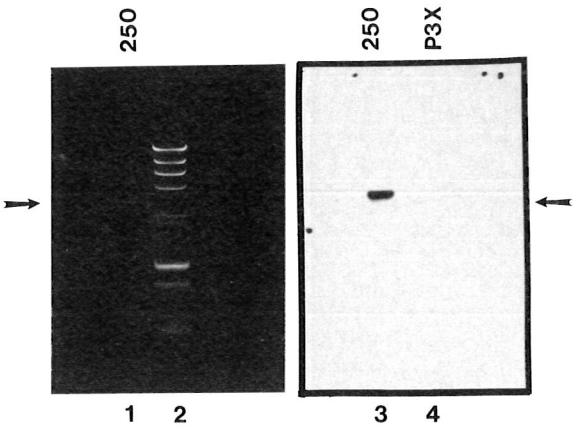


**Figure 2. TAb 250 $V_\kappa$ gene cloned by PCR.** Aliquots of the PCR reaction mix using primers designed to amplify the TAb 250 $V_\kappa$ region were analyzed on a 0.8% agarose gel (lanes 1-2) and by a Southern blot (3-4). Lane 1 and 3, the amplification from genomic DNA of TAb 250 hybridoma; Lane 2, DNA markers (see Figure 1); Lane 4, the amplification from genomic DNA of P3X cells. The amplified TAb 250 $V_\kappa$ DNA band is indicated by an arrow.

TAb 250 antibody perfectly match with those predicted from the nucleotide sequences of the cloned TAb 250 $V_H$ and $V_\kappa$ genes (data not shown). This evidence further confirmed the cloned TAb 250 $V_H$ and $V_\kappa$ genes.

Human $C_{\gamma-1}$ gene and human $C_\kappa$ gene were also cloned individually by PCR from cell line ARH-77. Both amplified $C_{\gamma-1}$ and $C_\kappa$ genomic DNA fragments were veri-

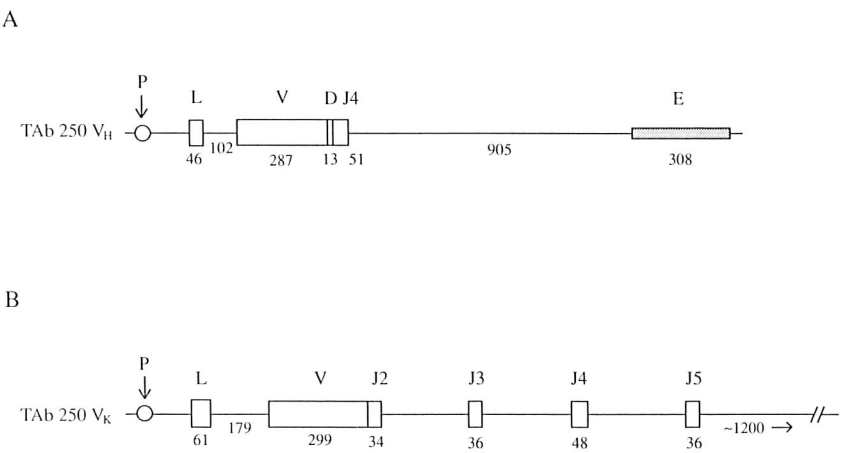


**Figure 3. Gene maps of cloned TAb 250 VH region and TAb 250 $V_\kappa$ region.** The functionally rearranged TAb 250 $V_H$ gene and $V_\kappa$ gene were mapped according to their nucleotide sequence information. Exons are represented by boxes and introns are represented by lines. The enhancer sequence is indicated by the shaded box. P, promoter; L, leader exon; V(D)J, Variable (diversity) Joining region exons; E, enhancer. The number of nucleotides in each region is indicated by the numbers.

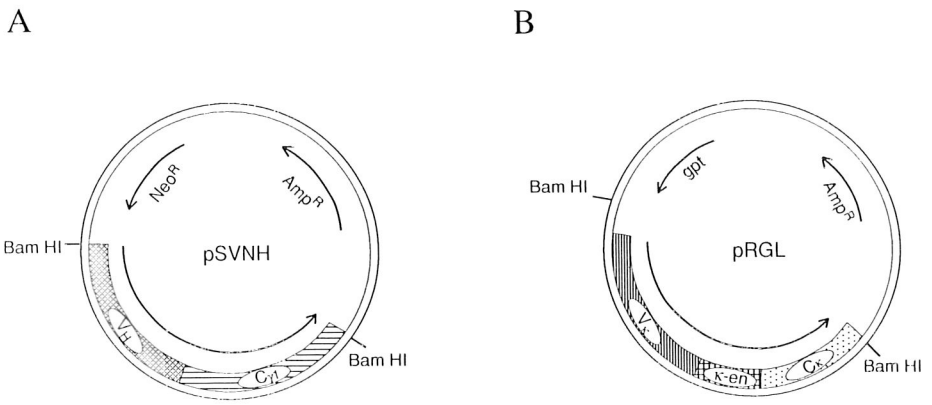


**Figure 4. Structures of chimeric heavy and light chain expression plasmids.** The chimeric heavy chain expression plasmid, pSVNH (4A), and the chimeric light chain expression plasmid, pRGL (4B), were constructed as described in Materials and Methods. The assembled chimeric heavy and light chain genes are represented by the blocks, and the variable and constant regions in the gene are indicated. The gene for ampicillin resistance (AmpR), the gene for G418 resistance (neo), and the xanthine-guanine phosphoribosyl transferase gene (gpt) are mapped in the plasmids. The orientation of genes in the plasmids is indicated by an arrow.

fied by Southern hybridization (data not shown). The TAb 250 $V_H$ gene and human $C_{\gamma-1}$ gene were combined and subcloned into vector pSV2-neo to construct the chimeric heavy chain expression plasmid pSVNH (Figure 4A). The TAb 250 $V_\kappa$ gene, mouse enhancer and human $C_\kappa$ gene were assembled and subcloned into vector pRSVgpt to construct the chimeric light chain expression plasmid pRGL (Figure 4B).

*Expression of and characterization of chimeric antibody.* The chimeric heavy and light expression plasmids were cotransfected into mouse myeloma cells and ELISA results indicated that positive clones produced both chimeric heavy chain and light chain at comparable levels. The molecular weight, assembly pattern and the secretion of BACh 250 were examined (Figure 5). The secreted BACh 250 chimeric antibodies were properly assembled indicated by a single band at a molecular weight of ~200 Kd present in the culture media. Only low levels of fully assembled antibody molecules accumulated inside the cells, indicating that the chimeric antibodies produced by the cells were properly secreted.

The ability of chimeric antibody BACh 250 to bind c-erbB-2 antigen was demonstrated in an ELISA using c-erbB-2-transfected NIH3T3 cells. BACh 250 and TAb 250 were further compared in a competitive binding assay. As shown in Figure 6, BACh 250 competed with $^{125}$I-TAb 250 for binding to c-erbB-2 antigen on the surface of SKBR-3 cells and 80% of $^{125}$I-TAb 250 bound was displaced by BACh 250 at a concentration of 10 μg/ml. TAb 250 competed $^{125}$I-TAb 250 at comparable concentrations. These results indicated that BACh 250 retains the same binding specificity and affinity as its parent hybridoma TAb 250.

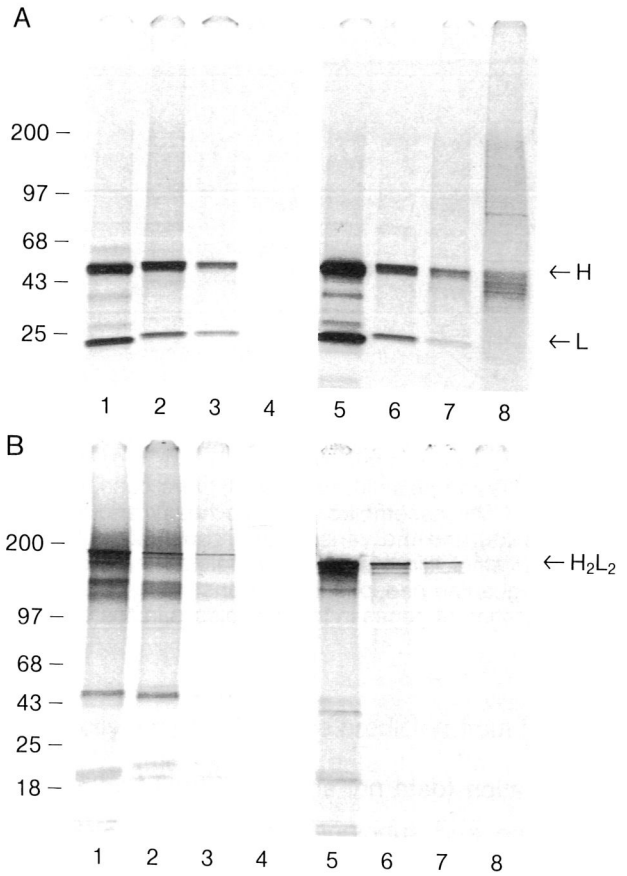


**Figure 5. Analysis of chimeric antibody BACh 250.** BACh 250 or TAb 250 was metabolically labeled, immunoprecipitated and analyzed on a SDS polyacrylamide gel under both reducing conditions (5A) and non-reducing conditions (5B). Lanes 1 and 5 represent parental hybridoma TAb 250; Lanes 2 and 6 represent BACh 250 subclone-1; Lanes 3 and 7 represent BACh 250 subclone-2; Lanes 4 and 8 represent recipient cell line P3X. Lanes 1-4 represent cytoplasmic antibody and Lanes 5-8 represent secreted antibody. Both BACh 250 subclones shown here were selected from P3X cell line. The protein molecular weight standards, heavy chain protein (H), light chain protein (L), and protein of assembled tetrameric molecules ($H_2L_2$) are indicated.

*Biological properties of chimeric antibody.* The effect of BACh 250 on cell proliferation was demonstrated in a cell proliferation assay (Figure 7). SKOV-3 cells treated with 10 μg/ml of BACh 250 showed a growth inhibition of 20% on Day 2, and 50% on Day 11 as compared to the control cells. Similar growth inhibition was observed in TAb 250 treated cells, suggesting the chimeric BACh 250 has the same antiproliferative effect on tumor cells expressing c-erbB-2 as the monoclonal TAb 250.

To assess whether the chimeric antibody has improved effector function attributed to the human γ1 constant region, TAb 250 and BACh 250 were compared in CDC and ADCC assays. (Figure 8). Figure 8A demonstrates specific lysis of SKBR-3

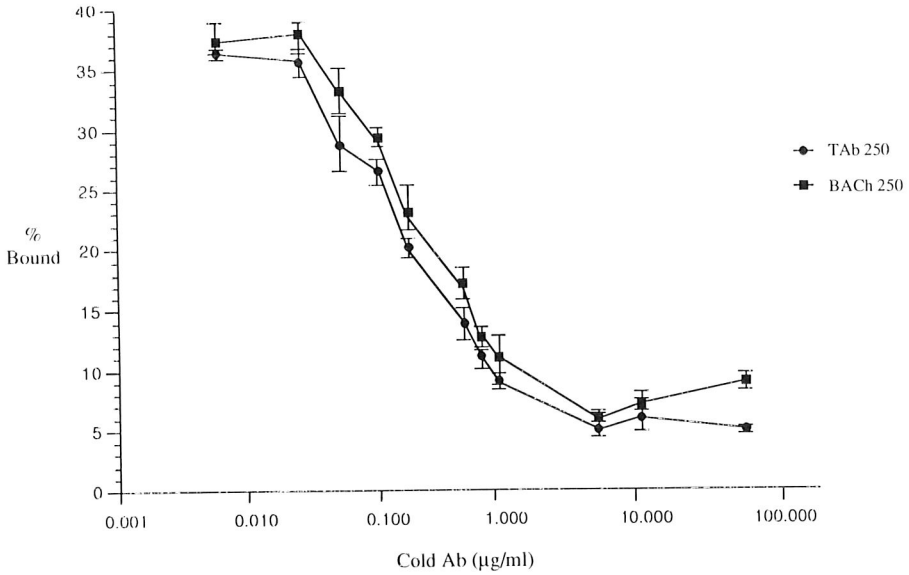


**Figure 6. Binding affinity of chimeric antibody BACh 250.** Competition of TAb 250 and BACh 250 with $^{125}$I-TAb 250 for binding to SKBR-3 cells was determined as described in Materials and Methods. Each point was determined in triplicate and bars represent standard deviations.

target cells after treatment with BACh 250 and rabbit complement at dilutions of 1:10 and 1:20 for 1 h. TAb 250, a murine $IgG_1$ antibody failed to mediate this lysis. This is consistent with previous reports that indicated murine $IgG_1$ antibodies are ineffective at

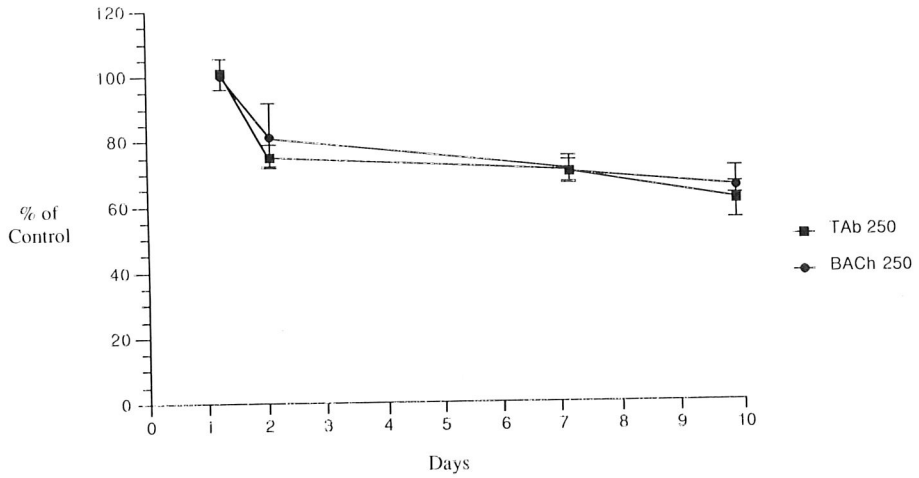


**Figure 7. The antiproliferative effect of BACh 250 on c-erbB-2-expressing tumor cells.** The ability of BACh 250 to inhibit the growth of SKOV-3 cells *in vitro* was determined using a cell proliferation assay. Closed circles represent inhibitory effects of chimeric BACh 250 while the closed squares represent the inhibitory effects of the parental TAb 250. Each point is the mean of triplicate determinations and bars represent the standard deviations.

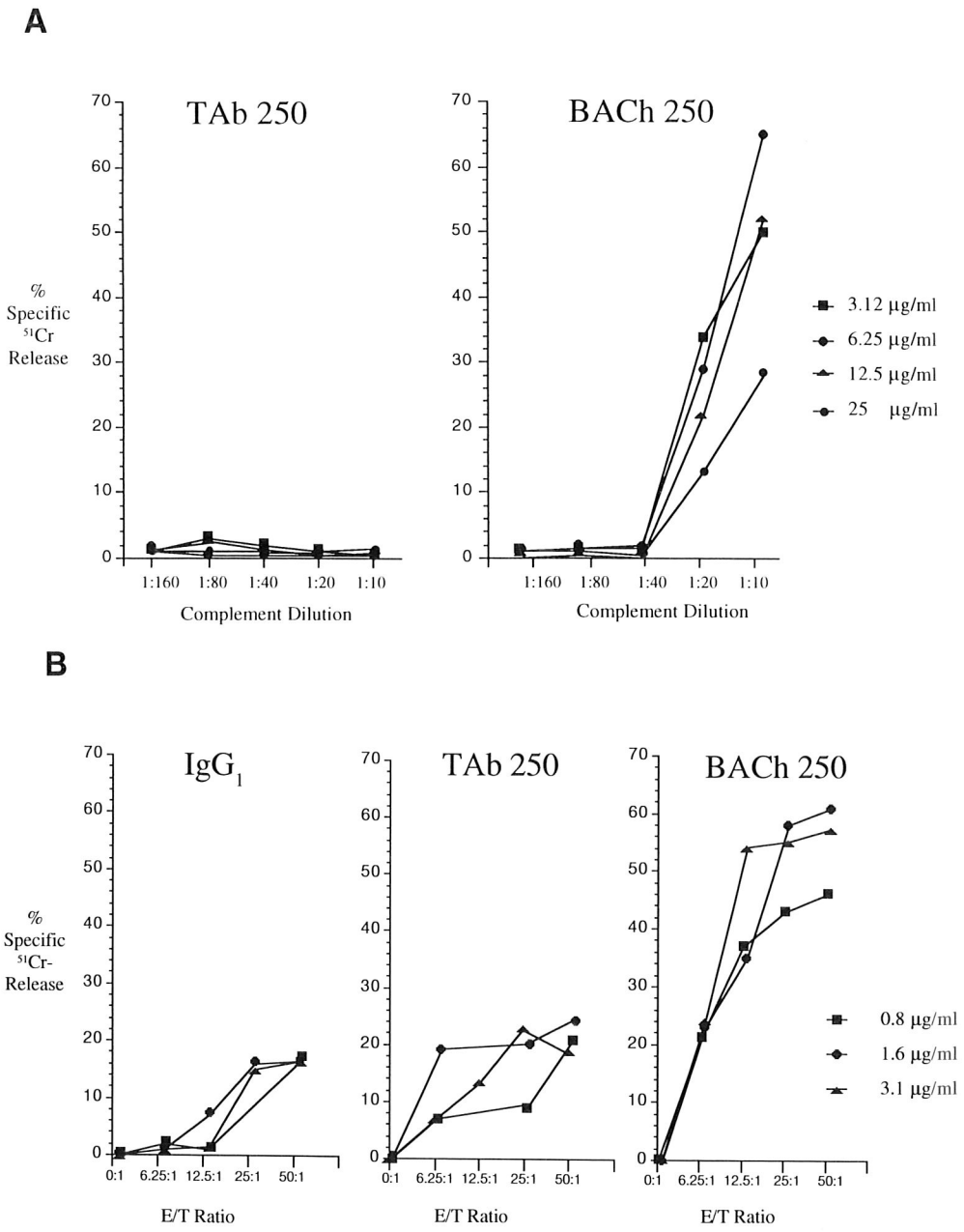


**Figure 8. Ability of BACh 250 to mediate immune effector functions.** For complement mediated cytotoxicity (A), TAb 250 or BACh 250 (filled square, 3.12 µg/ml, filled circle, 6.25 µg/ml) was added to $^{51}$Cr-labeled SKBR-3 cells in the presence of rabbit complement at the dilutions indicated. The cells were incubated at 37°C for 1 h and supernatants were harvested and counted in a gamma counter. For Antibody Dependent Cellular Cytotoxicity (B), human effector cells (PBMC) isolated by density gradient centrifugation were added to $^{51}$Cr-labeled SKBR-3 target cells at various effector to target (E:T) ratios in the presence of either an IgG$_1$ isotype control, TAb 250 or BACh 250 (filled square, 0.8 µg/ml; filled circle, 1.6 µg/ml; filled triangle, 3.1 µg/ml). Cells were coincubated for 24 h at 37°C, supernatants were harvested, counted in a gamma counter. Percent specific release was calculated as described in Materials and Methods.

mediating complement-dependent lysis. The complement-mediated lysis effected by BACh 250 was specific to c-erbB-2- expressing cells as MDA-MB-468, a tumor cell line which does not express c-erbB-2, were not lysed (data not shown).

In ADCC assays (Figure 8B), BACh 250 mediated an antibody-dependent lysis compared to cells treated with either TAb 250 or an $IgG_1$ isotype control antibody at E:T ratios of 12.5:1 to 50:1. TAb 250 mediated lysis of 8-22% of the cells at all of the concentrations tested. This lysis is not significantly greater than that of the control antibody except at one E:T ratio of 6.25:1. In contrast, target cell lysis achieved with BACh 250 in the presence of peripheral blood mononuclear cells at E:T ratios of 25-50:1 was approximately 40-60% which was significantly greater than that observed for TAb 250 at E:T ratios of 12.5-50:1. The lysis mediated by BACh 250 in ADCC assay was specific, as MDA-MB-468 cells were not lysed (data not shown).

TAb 250 has been previously shown to inhibit growth of SKOV-3 xenografts by 20-30% and the inhibition increased to 80-95% when TAb 250 was used in conjunction with Cisplatin (23). In order to assess whether the chimera is also able to inhibit tumor growth, an *in vivo* experiment was performed to compare TAb 250 and BACh 250, either alone or in combination with Cisplatin (CDDP). In animals treated with TAb 250, BACh 250 or CDDP alone, tumor growth was inhibited 40-50% as compared to $IgG_1$ isotype control (Figure 9). In contrast to single agent treatment, a marked tumor growth inhibition of 90% was seen in animals treated with a combination of either TAb 250 and CDDP or BACh 250 and CDDP. In these groups, two out of eight animals in the TAb 250 + CDDP group and three out of eight animals in the BACh 250 + CDDP group showed no tumor growth at the end of the experiment. In comparison, all animals in the control group developed substantial tumor nodules.

## DISCUSSION

Previous mouse/human chimeric antibody constructions have used either genomic gene fragments (20, 26-29) or c-DNA fragments (30-33). These procedures frequently involved preparation of DNA libraries and subsequent laborious screening. The polymerase chain reaction (PCR) technique has also been introduced to obtain cDNA and promoterless genomic DNA fragment of the antibody variable regions in chimeric antibody construction (30, 32,34). In both cases, transcription and regulatory elements have to be provided by expression vectors for gene expression. The published genomic PCR method has a prerequisite of identifying the exact sequence of 5'-untranslated regions and the rearranged J segments of heavy and light chains from each antibody, which involves a long and complicated procedure.

In this work, we have demonstrated a PCR approach to rapidly clone the genomic sequences of immunoglobulin variable genes from genomic DNA of mouse hybridoma cells secreting the specific monoclonal antibody. The PCR primers were designed to include the upstream heavy and light gene promoter elements, signal sequences and enhancers in the same fragments as the variable gene coding regions

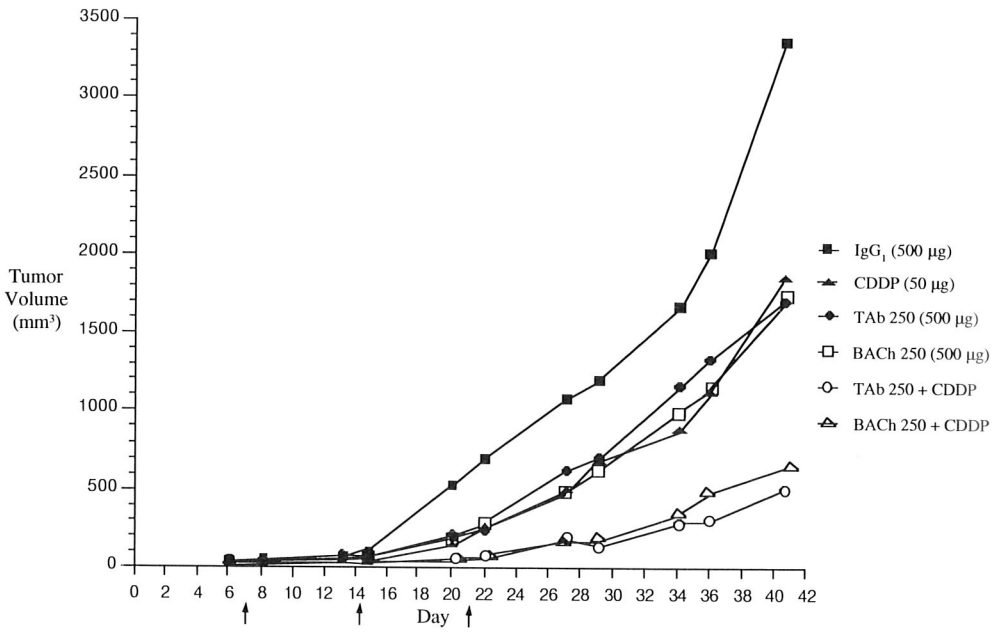


**Figure 9. Effect of BACh 250 and CDDP on SKOV-3 tumor xenografts.** Female BAlb/c nu/nu mice (n = 8 animals per group) were implanted subcutaneously with freshly processed SKOV-3 passaged tumors (as described in Materials and Methods). Treatments were administered IP on day 7, 14 and 21 after tumor implant and tumors were measured 2x/wk using vernier calipers. Volume was calculated as the product of L x W x H. Animals were treated with either an isotype control antibody $IgG_1$ (filled square), TAb 250 (filled circle), BACh 250 (open square), CDDP (filled triangle), or the combination of TAb 250 + CDDP (open circle) or BACh 250 + CDDP (open triangle). Antibody was administered at 500 µg/dose and CDDP at 50 µg/dose. The curve for the group treated with the combination of $IgG_1$ + CDDP is not shown as results were similar to the effects seen for group treated with CDDP alone.

for later chimeric immunoglobulin gene expression. We have successfully amplified both $V_H$ and $V_\kappa$ genes and shown that they were the rearranged heavy and light chain genes from TAb 250. The chimeric heavy chain and light chain proteins were synthesized, processed and properly assembled into tetrameric molecules in mouse myeloma cells, demonstrating that the upstream promoter region sequences amplified with our PCR primers are sufficient to promote efficient gene expression. The oligonucleotide PCR primers used for TAb 250 $V_H$ and $V_\kappa$ cloning were designed to cover all available mouse immunoglobulin variable sequences. Therefore, it is reasonable to predict that these primers can be used in PCR cloning of variable region genomic genes for any mouse monoclonal antibody.

The chimeric antibody, BACh 250, retained the same binding affinity to the c-erbB-2 antigen. It also exhibited comparable inhibitory effects on tumor cell growth comparable to the parental mouse monoclonal antibody, both *in vivo* and *in vitro*. In addition, the human Fc region of BACh 250 interacts more efficiently with human cells to mediate tumor destruction as shown by its enhanced ability to mediate complement-

dependent and cell-mediated cytotoxicity *in vitro*. Human constant region domains, which may reduce immunogenicity in humans, along with the demonstrated efficacy in an *in vivo* tumor model make BACh 250 attractive as a potential therapeutic candidate.

## REFERENCES


1. King, C.R., Kraus, M.H., and Aaronson, S.A. (1985) Science 229: 974-76.
2. Semba, K., Kamata, N., *et al.* (1987) Proc. Natl. Acad. Sci. 82: 6497-501.
3. Kraus, M.H., Popescu, N.C., *et al.* (1987) J. EMBO 6: 605-10.
4. van de Vijver, M., van de Bersselaar, *et al.* (1987) Mol. Cell Biol. 7: 2019-23.
5. Slamon, D.J., Clark, G.M., *et al.* (1987) Science 235: 177-82.
6. Yokota, T., Yamamoto, T., *et al.* (1988) Oncogene 2: 283-7.
7. Natali, P. G., Nicotra, M.R., *et al.* (1990) Int. J. Cancer 45: 457-61.
8. Meeker, T.C., Lowder, *et al.* (1985) Blood 65: 1349-63.
9. Schroff, R.W., Foon, K.H., *et al.* (1985) Cancer Res. 45: 879-85.
10. Shawler, D.L., Bartholomew, R.M., *et al.* (1985) J. Immunol. 135: 1530-35.
11. Courtenay-Luck, N.S., Epenetos, A.A., *et al.* (1986) Cancer Res. 46: 6489-93.
12. Miller, R.A., Lowder, *et al.* (1987) Natl. Cancer Inst. Monogr. 3: 131-34.
13. Morrison, S.L., Johnson, M.J., *et al.* (1984) Proc. Natl. Acad. Sci. USA 81:6851-55.
14. Boulianne, G.L., Hozumi, N., and Shulman, J.J. (1984) Nature 312: 643-46.
15. Neuberger, M., Williams, G., *et al.* (1985) Nature (London) 314: 268-70.
16. Morrison, S. (1985) Science 229: 1202-07.
17. Liu, A.Y., Robinson, R.R., *et al.* (1987) Proc. Natl. Acad. Sci. USA 84: 3439-43.
18. Liu, A.Y., Robinson, R.R., *et al.* (1987) J. Immunol. 139: 3521-26.
19. Nishimura, Y., Yokoyama, M., *et al.* (1987) Cancer Res. 47: 999-1005.
20. Hamada, H., Miura, K., *et al.* (1990) Cancer Res. 50: 3167-71.
21. Langton, B.S., Crenshaw, M.C., *et al.* (1991) Cancer Res. 51: 2593-98.
22. Devereux, J., Haeberli, P., and Smithies, O. (1984) Nucleic Acid Res. 12(1): 387-95.
23. Hancock, M.C., Langton, B.C., *et al.* (1991) Cancer Res. 51: 4575-80.
24. Shawver, L.K., Mann, E., *et al.* (1994) Cancer Res. 54:1367-73.
25. Current Protocols in Immunology, Vol.2, (1992) ed. J.E. Coligan et al., John Wiley & Sons.
26. Sun, L. K., Curtis, P., *et al.* (1987) Proc. Natl. Acad. Sci. USA 84: 214-18.
27. Beidler, C.B., Ludwig, J.R., *et al.* (1988) J. Immunol. 141: 4053-60.
28. Kameyama, K.-Z., Imai, *et al.* (1989) Febs Letters 244: 301-6.
29. Neumaier, M., Shively, L., *et al.* (1990) Cancer Res. 50: 2128-3.
30. Orlandi, R., Güssow, *et al.* (1989) Proc. Natl. Acad. Sci. USA 86: 3833-37.
31. Gillies, S.D., Lo, K.-M. and Wesolowski, J. (1989) J. of Immunol. 125: 191-202.
32. Larrick, J.W., Danielsson, L., *et al.* (1989) Biotech. 7: 934-38.
33. Hoogenboom, H.R., Raus, J.C.M., and Volckaert, G. (1990) J. of Immunol. 144: 3211-17.
34. Weissenhorn, W., Weiss, E., *et al.* (1991) Gene 106: 273-277.