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Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells

(Immunoglobulin domain cDNA/DNA transfection/tumor antigen/complement-dependent cytolysis/antibody-dependent cellular cytotoxicity)


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Communicated by Paul D. Boyer, January 28, 1987 (received for review December 22, 1986)

ABSTRACT A chimeric mouse-human antibody has been created that recognizes an antigen found on the surface of cells from many carcinomas. Immunoglobulin constant (C) domains of the mouse monoclonal antibody L6, C\textsubscript{\textgamma 2a} and C\textsubscript{\textgamma 4}, were substituted by the human C\textsubscript{\textgamma 2a} and C\textsubscript{\textgamma 4} by recombing cDNA modules encoding variable or C domains. The cDNA constructs were transfected into lymphoid cells for antibody production. The chimeric antibody and mouse L6 antibody bound to carcinoma cells with equal affinity and mediated complement-dependent cytolysis. In the presence of human effector cells, the chimeric antibody gave antibody-dependent cellular cytotoxicity at 100 times lower concentration than that needed for the mouse L6 antibody. The chimeric antibody, but not the mouse L6 antibody, is effective against a melanoma line expressing small amounts of the L6 antigen. The findings point to the usefulness of the chimeric antibody approach for obtaining agents with strong antitumor activity for possible therapeutic use in man.

The presence of tumor-associated antigens at the cell surface is a characteristic of many cancers. Since these antigens are either absent or found in much lower amounts in normal cells, it should be possible to use antibodies for targeting of tumors. A sizable collection of relatively tumor-specific monoclonal antibodies (mAb) of mouse origin is available (1). Some of these mAb possess tumoricidal activity in the presence of human effector cells [antibody-dependent cellular cytotoxicity (ADCC)] or serum [complement-dependent cytolytocity (CDC)] (2, 3). It has been shown (4) that partial tumor regression can be achieved when mAb possessing such functional activity are given to patients. One complication preventing repeated use of mouse mAb in man is that they are immunogenic. Furthermore, mouse mAb may interact less efficiently with human effector cells to mediate tumor destruction.

A method made possible by recombinant DNA technology was chosen to generate chimeric mouse-human antibodies. It entails the replacement of the mouse constant (C) domain regions with the corresponding human equivalents (5–7). In this study we have generated a mouse-human chimeric L6 antibody in which the mouse constant domains C\textsubscript{\textgamma 2a} and C\textsubscript{\textgamma 4} are substituted by the human C\textsubscript{\textgamma 2a} and C\textsubscript{\textgamma 4}. First, the cDNAs encoding the immunoglobulin genes were isolated. Next, restriction enzyme recognition sites were created in the cDNA sequences at the V/C junction (where V stands for variable) (9) by in vitro mutagenesis using oligodeoxynucleotides (10). The chimeric cDNAs thus constructed were then introduced into lymphoid cells by DNA transfection. The chimeric antibody isolated from the transfectants was compared with the mouse L6 for effector functions.

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MATERIALS AND METHODS

DNA Transfection of Mouse Sp2/0 Lymphoid Cells. Expression plasmid pING2114 (50 µg), linearized at a unique site (Aat II) in the nonessential bacterial region (see Fig. 3A), was transfected into 10^7 mouse Sp2/0 cells (CRL 1581, ATCC) by electroporation (11, 12). Transformants were selected in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum (HyClone, Logan, UT) and G418 at 0.8 mg/ml (GIBCO). The transfection frequency was between 10^-5 and 10^-4. Human antibody in the medium was detected by ELISA (13).

Isolation of Chimeric Antibody. Antibody-producing cells were grown to a density of 10^6 cells per ml and then shifted to serum-free DMEM 24 hr before harvest. Antibody secreted by the cells was concentrated by ultrafiltration, then chromatographed on a DEAE-cellulose column equilibrated in 40 mM NaCl/10 mM sodium phosphate, pH 8.0. The antibody in the flow-through was further purified to apparent homogeneity on protein A-Sepharose (14). For production of ascites fluid, 10^6 cells were injected into pristane-primed BALB/c mice. The chimeric antibody was purified by anti-human IgG-Sepharose chromatography (14).

Functional Tests of the Chimeric L6 Antibody. The following tests were included: (i) measurement of antibody binding to target cells, either positive or negative for reactivity with the mouse L6; (ii) competitive inhibition of binding of L6 to these cells; (iii) assays for CDC and ADCC. The binding tests were performed using a Coulter model EPIC-C cell sorter (8). The assays for CDC and ADCC were carried out on ^51Cr-labeled target cells (2, 3) that were exposed to antibodies and human serum or peripheral blood leukocytes over a 4-hr period.

Abbreviations: V, variable; C, constant; J, joining; mAb, monoclonal antibodies; CDC, complement-dependent cytolytocity; ADCC, antibody-dependent cellular cytotoxicity; SV40, simian virus 40; H, heavy.

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Fig. 1. Nucleotide sequences and predicted amino acid sequences of the L6 VH (A) and VL (B). The framework (FR) and complementarity determining region (CDR) segments are indicated. The diversity (D) segment in VH is underlined. Circles above the amino acid sequences of the L6 VH and VL are labeled with the relevant FR and CDR numbers. The sequence is present in plasmid pH3-6a. The CDR3 at the 5' end is removed by oligonucleotide-mediated mutagenesis. The VH sequence is present in plasmid pH3-6a. The CDR3 at the 5' end is removed by oligonucleotide-mediated mutagenesis. The VH sequence is present in plasmid pH3-6a. The CDR3 at the 5' end is removed by oligonucleotide-mediated mutagenesis. The VH sequence is present in plasmid pH3-6a. The CDR3 at the 5' end is removed by oligonucleotide-mediated mutagenesis.
RESULTS

Isolation of Mouse cDNA. A cDNA library was generated from the L6 hybridoma cells by priming poly(A)+ RNA with oligo(dT) as described (9, 15). The probes used to screen the library were a J5 oligonucleotide, d(GTCCCGAACAGCAGGCAAG), for the light chain and a JH2 oligonucleotide, d(TGGCTGGAGAGACTGGTAGAG) for the heavy chain (where J stands for joining and H stands for heavy). Two methods (16, 17) were used to determine that the L6 x mRNA contains J5 sequences and that the L6 yA mRNA contains J2 sequences.

Preparation of Mouse V-Region cDNA Modules. Restriction enzyme sites were engineered into the immunoglobulin cdNA around the V/C border for recombining mouse V regions to human C modules. The oligonucleotide MIIIH2ApaI [d(ATGGCCCTTTGTGCTGGCTGAGGAGACTGT)] (with the restriction enzyme site underlined) was used for mutagenesis of the V\(\gamma\) cDNA; and the oligonucleotide JxHindIII [d(CTCAAGCTTGGTCCCAGT)] for that of the V\(\gamma\) segment. The oligonucleotide described above was used to generate a Sal I site eight residues on the 5' side of the V\(\gamma\), ATG codon. By cleaving with Sal I the oligo(dGC) segment on the 5' side of the cDNA insert was removed. The oligo(dGC) segment on the 5' side of the V\(\gamma\) cDNA, the nuclear sequence, was used. The digested products were inserted into the vector pGML60, containing regulatory sequences derived from the plasmid pLl (19) in such a way that the Ml3 restriction site was engineered into the immunoglobulin M13mp19 (19) in such a way that the Ml3 restriction site was placed upstream of the SV40 promoter (9). The selectable marker is the Tns neo gene that confers resistance to the drug G418.

The heavy-chain plasmid pLNG211 was constructed by first joining the mouse V\(\gamma\) cDNA module in a Sal I-Apa I DNA fragment with the human C\(\gamma\) cDNA module in an Apa I-Apa I DNA fragment. The ligated fragments were then inserted into pLNG2012E cleaved by Sal I-BamHI. The light-chain plasmid pLNG219 was constructed by joining the mouse V\(\gamma\), cDNA module in a Sal I-HindIII DNA fragment with the human C\(\gamma\), cDNA module in a HindIII-BamHI DNA fragment. The same vector fragment was used (Fig. 3A). In both plasmids the cDNA gene is placed 11 nucleotide residues downstream of the SV40 19S 3'-splice acceptor (9). The cDNA ends in a segment approximately 100 G+G, where it is joined to the SV40 transcription-termination/polyadenylation sequences. Fig. 3B shows the incident nucleotide sequence changes made at the V/C junction as a result of the gene construction.

A two-gene plasmid, pLNG2114, was constructed from pLNG211 and pLNG219 in which the light-and heavy-chain gene transcription units are in tandem (Fig. 3A). By using this plasmid, we introduced an equal ratio of heavy- and light-chain genes into recipient cells. Unexpectedly, we observed that there was a consistently higher expression of heavy than of light chain in all transfected cell lines examined (data not shown). The two transcription units differ in that the light-chain gene is about 700 base pairs shorter than the heavy-chain gene, and the C\(\gamma\), segment has a higher A+T content. This imbalance was reduced by introducing more light-chain gene copies carried on a second plasmid with a different selectable marker [pLNG2121a, an Eco-gpt (22) version of pLNG2119].

Two initial Sp2/0 transformants, D7 and 3E3, obtained by transfection with pLNG2114 were cultured for the isolation of chimeric antibody. D7 secretes 10% of the antibody produced by 3E3—x (17 \mu g/liter) and y (77 \mu g/liter) chains for D7 compared to x (100 \mu g/liter) and y (700 \mu g/liter) chains for 3E3.

Binding Characteristics of Chimeric Versus Mouse L6 Antibody. Table 1 shows that the chimeric L6 antibody binds to cells from a human colon carcinoma (line C-3347) that expresses 5 x 10^6 molecules per cell of the antigen defined by the mouse L6 mAb (8). In a competition assay, 50% inhibition of binding was achieved by the same amount of the chimeric mouse L6 (Fig. 4). Cells from a T-cell line, HSB-2, did not bind either mouse L6 or the chimeric antibody. Data on the melanoma line M-2669, clone 13 (3), are also included in Table 1, since this line, which expresses a low level of the L6-defined antigen, was used for the functional studies (see below).

Chimeric L6 Antibody Mediates CDC and ADCC. Fig. 5 shows that both the chimeric and mouse L6 antibodies lysed tumor cells in the presence of human complement. The experiment further showed that the chimeric L6 gave higher CDC at all dilutions of the complement.
before fluorescein isothiocyanate-conjugated mouse L6 (3 µg/ml) was added. Antibody inhibition assays, performed by fluorescence-activated cell sorting. C-3347 cells were incubated with the blocking antibodies because ADCC was not observed with the following three cell lines lacking detectable L6 antigens: B-cell lines DHL-10 (data not shown) and the T-cell line HSB-2 (as bright as the control). A ratio of 2 means that the test sample is as bright as the control, NT, not tested.

**DISCUSSION**

The mouse mAb L6 recognizes a carbohydrate antigen present in abundance in a variety of carcinomas. Normal tissues express only trace amounts of the antigen. Based on this specificity there is justification in considering L6 for cancer treatment with the mAb used either alone (2) or as a carrier of anticancer agents. However, the immunogenicity of mouse L6 mAb in man is a disadvantage for its sustained use in patients, and its functional activity (ADCC and CDC) may be insufficient to effect optimal tumor destruction at the concentration, µg/ml | Binding ratio* |
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*The binding ratio is the number of times a test sample is brighter than a control sample when treated with GAM (fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin) or with GAH (fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin). For example, a ratio of 1 means that the test sample is as bright as the control; a ratio of 2 means that the test sample is twice as bright as the control. NT, not tested.
We show here that this is a useful approach to improve ADCC by the mouse L6. In patients one may speculate that the chimeric L6 would remain longer in the circulation. This, in combination with the functional attributes of chimeric L6, should make it a strong candidate for therapeutic trials. Some of the antibodies induced in man to mouse mAb were directed to idiotypic determinants (26, 27). It remains to be seen whether the immunogenicity of those determinants of the chimeric L6 will be different from that of the mouse L6.

The advantage of the cDNA approach lies in the ease with which immunoglobulin gene cDNAs can be isolated. The technology used for the present work should make it possible to convert many other mouse mAb to chimeric antibodies with improved antitumor activity via ADCC and CDC mechanisms. The chimeric antibodies will augment the relatively few human mAb currently used in the treatment of cancer (28).

We thank Cathy Shapiro, Phil Mack, Phil Mixter, Pam Smith, Susan Azemove, Grethe Lovold, and Pat McGowan for excellent technical assistance. We also thank Randy Wall for discussion, and Randy Wall, Carol Hersh, Arup Sen, Gary Wilcox, Perry Fell, Jeff Ledbetter, Peter Linsley, and Erik Milner for useful comments on the manuscript. The work was supported by INGENE and ONCOGEN.


**Fig. 6.** (A) Titration of chimeric and mouse L6 antibodies in ADCC assays with human peripheral blood leucocytes. E/T, effector-target cell ratio. Two preparations of chimeric L6 were used. (B) Titration of human peripheral blood leucocyte effector cells mediating ADCC in the presence of antibodies (2.5 μg/ml). (C) Titration of L6 (chimeric mouse) in ADCC assays on the DHL-10 T-cell line. 1F5 is a mouse mAb that recognizes the DHL-10 cells.