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Immunology

Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains

(transfection/protoplast fusion/calcium phosphate transfection/intronic controlling elements/transfectoma)

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ABSTRACT **We have created mouse–human antibody molecules of defined antigen-binding specificity by taking the variable region genes of a mouse antibody-producing myeloma cell line with known antigen-binding specificity and joining them to human immunoglobulin constant region genes using recombinant DNA techniques. Chimeric genes were constructed that utilized the rearranged and expressed antigen-binding variable region exons from the myeloma cell line S107, which produces an IgA (κ) anti-phosphocholine antibody. The heavy chain variable region exon was joined to human IgG1 or IgG2 heavy chain constant region genes, and the light chain variable region exon from the same myeloma was joined to the human κ light chain gene. These genes were transfected into mouse myeloma cell lines, generating transformed cells that produce chimeric mouse–human IgG (κ) or IgG (κ) anti-phosphocholine antibodies. The transformed cell lines remained tumorigenic in mice and the chimeric molecules were present in the ascitic fluids and sera of tumor-bearing mice.**

The capability to transfer immunoglobulin genes into lymphoid cells where they produce protein in quantities sufficient for structural studies (1–3) provides us with the opportunity to generate and characterize novel immunoglobulin molecules. Cloned variable (*V*) region genes from mouse or rat hybridoma cell lines can be ligated to human constant (*C*) region genes and we would expect that these chimeric genes can be transfected into mouse myeloma cells, which then will produce novel human antibody molecules. We would thus produce antibodies that are largely human but which have antigen-binding specificities generated in mice. The additional potential for *in vitro* manipulation and alteration of both the antigen-binding site and the structures correlated with biological effector functions of these antibody molecules using recombinant DNA techniques would introduce a powerful approach for further understanding antibody structure, function, and immunogenetics.

As we show here, both chimeric mouse heavy chain *V* region exon (V_H)–human heavy chain *C* region genes and chimeric mouse light chain *V* region exon (V_κ)–human κ light chain gene constructs are expressed when transfected into mouse myeloma cell lines. When both chimeric heavy and light chain genes are transfected into the same myeloma cell, an intact tetrameric (H_2L_2) chimeric antibody is produced. In this study we used V_H and V_κ exons from the mouse phosphocholine (*P*Cho)-binding antibody-producing S107 myeloma cell line (4, 5). Chimeric mouse–human anti-*P*Cho antibodies were produced in culture by appropriate transfected cell lines or by "transfectomas" obtained when such cell lines are injected into mice.

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MATERIALS AND METHODS

Chimeric Genes. The cloned S107 V_H and S107 V_κ genes were gifts from Matthew Scharff (Albert Einstein College of Medicine, Bronx, NY). The S107 V_H gene was spliced to human IgG1 and IgG2 *C* region genes by using *Sal* I linkers as shown in Fig. 1*A*. Both constructs were inserted into the vector pSV2ΔH-gpt (1, 6). The S107 V_κ gene was spliced to the human κ gene at a unique *Hin*dIII site located in the large intron between the κ light chain joining and *C* (J_κ and C_κ) region exons as shown in Fig. 1*B*. This chimeric light chain gene construct was inserted into both PSV2ΔH-gpt and pSV2-neo plasmid vectors (7).

Transfection. Protoplast fusion and calcium phosphate precipitation techniques (1, 8, 9) were used to transfect these chimeric immunoglobulin genes into the J558L myeloma cell line (a λ light chain-producing mouse myeloma cell line) and an immunoglobulin nonproducing derivative of the P3 myeloma cell line. Mycophenolic acid (GIBCO) was used for selection of cells transfected with pSV2Δ-gpt vectors as described (1, 3). G418 (GIBCO) at 1.0 mg/ml was used for selection of cells transfected with pSV2-neo vectors (7).

Light and heavy chain chimeric immunoglobulin genes were transfected sequentially by protoplast fusion using G418 selection for the chimeric light chain gene vector and mycophenolic acid for the chimeric heavy chain gene vector. The protoplast fusion transfection procedure used was as described (1).

Transfection using the calcium phosphate precipitation procedure was done by transfecting a mixture of 40 μg each of the chimeric light and chimeric heavy chain pSV2ΔH-gpt vectors into 5×10^6 cells. Mycophenolic acid was used to select for transformed cell lines as described (1).

Antigen Binding. *P*Cho binding of antibody secreted into the culture supernates of transfected cell lines was analyzed by using a solid-phase radioimmunoassay described previously (10). *P*Cho-keyhole limpet hemocyanin antigen was bound to 96-well polyvinyl plates; binding of chimeric anti-*P*Cho antibodies was detected by using ^{125}I-labeled protein A or ^{125}I-labeled-anti-human IgG. *P*Cho-binding antibodies in biosynthetically labeled culture supernates and cell lysates of transfected cell lines also were analyzed by binding the biosynthetically labeled antibody to *P*Cho-coupled Sepharose 4B (Pharmacia) and then eluting the bound antibody with *P*Cho-hapten. The bound and eluted antibody was examined by $NaDodSO_4$/polyacrylamide gel electrophoresis ($NaDodSO_4$/PAGE). Biosynthetic-labeling procedures were as described (11).

Idiotope Analysis. Three hybridoma anti-idiotope antibodies, also kindly provided by Matthew Scharff and Angela

Abbreviations: V, variable; C, constant; J, joining; H, heavy chain; light chain; PCho, phosphocholine; kb, kilobase(s); NEPHGE

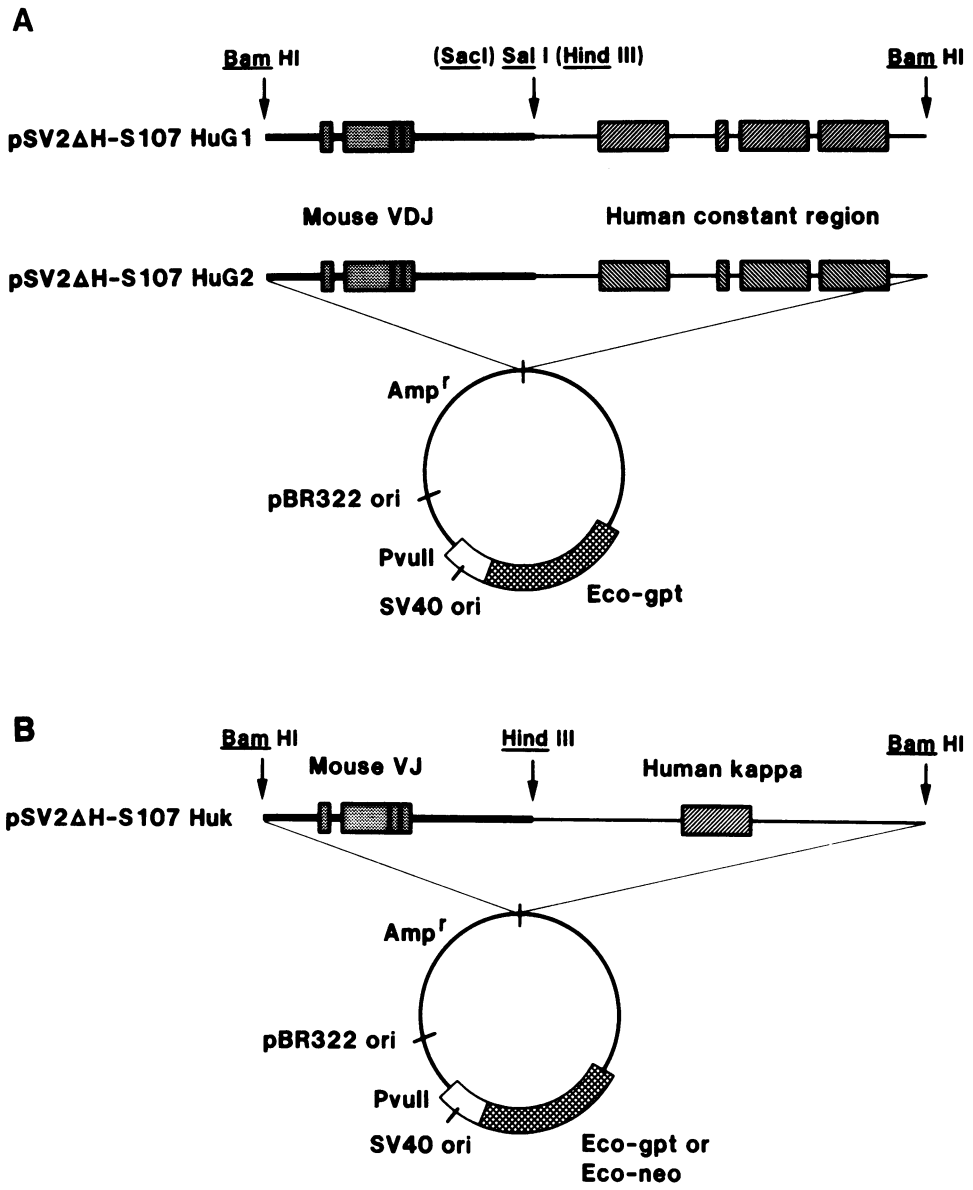

FIG. 1. Schematic diagrams (not drawn to scale) of the chimeric mouse–human heavy chain gene vector (*A*) and the chimeric light chain gene vectors (*B*). DNA fragment sizes are as follows: *Hin*dIII–*Bam*HI human IgG1 (HuG1) or IgG2 (HuG2) heavy chain gene, 7 kilobases (kb); mouse S107 V_H gene, 4.5 kb; *Hin*dIII–*Bam*HI human C_κ gene (HuK), 10 kb; and mouse S107 V_κ gene, 3.5 kb. The *Hin*dIII sites in the human IgG1 and IgG2 heavy chain genes were ligated to the *Sac* I site of the mouse S107 V_H gene with *Sal* I linkers.

Guisti (Albert Einstein College of Medicine), were used to analyze the V_H–V_L (L is light chain) domain structure of the chimeric human anti-*P*Cho antibodies. These antibodies (TC102.1.2, T139.2, and T156.1.1), recognizing three independent idiotopes (12), were used to immunoprecipitate biosynthetically labeled material eluted with *P*Cho from the *P*Cho-Sepharose 4B matrix. Immunoprecipitates were analyzed by $NaDodSO_4$/PAGE.

Immunoglobulin Chain Composition. Monoclonal anti-human IgG and anti-human κ antibodies (Becton–Dickinson) were used to immunoprecipitate biosynthetically labeled chimeric human anti-*P*Cho antibodies for analyses using two-dimensional nonequilibrium pH gradient PAGE (NEPHGE) (11, 13). *P*Cho-coupled Sepharose 4B also was used for affinity purification.

Immunoglobulin Heavy Chain Glycosylation. Tunicamycin (Calbiochem–Behring) was used to inhibit asparagine-linked glycosylation of biosynthetically labeled antibody from mouse cell lines producing mouse–human chimeric immunoglobulins (11). *P*Cho-binding antibody from tunicamycin-treated cells was analyzed by NEPHGE. Procedures for tunicamycin treatment were as described (11).

Chimeric Mouse–Human Antibody Production in Mice. Transfected J558L cells producing chimeric mouse–human antibody were injected intraperitoneally into BALB/c mice (10^6 cells per mouse). Sera and ascitic fluids from tumor-bearing mice were analyzed for human anti-*P*Cho antibody by a solid-phase radioimmunoassay (10) and by immunoelectrophoresis using a polyclonal anti-human IgG antiserum.

RESULTS

We obtained expression of chimeric mouse *V* region–human *C* region genes in transfected J558L and the immunoglobulin nonproducing P3 myeloma cell lines. When both light chain and heavy chain chimeric genes (see Fig. 1) were expressed in the same cell, tetrameric (H_2L_2) antigen-binding antibodies were obtained. Biosynthetically labeled antibody molecules secreted by J558L cells expressing chimeric genes were bound and hapten-eluted from *P*Cho-Sepharose 4B. Autoradiograms of two-dimensional NEPHGE analyses

pected charge and relative molecular mass. Identical two-dimensional gel analysis results were obtained with immunoprecipitates by using monoclonal anti-human κ and IgG antibodies. Similar results were obtained when the chimeric IgG2 (κ) antibodies produced in the transfected nonproducing P3 cell line were analyzed. Since the recipient P3 cell line does not produce endogenous immunoglobulin polypeptide chains, only the chimeric mouse–human heavy and light chains were seen (data not shown). *P*Cho binding by the chimeric antibody produced in the J558L cell line required the specific association of both the V_H and V_L domains of the S107 myeloma protein connected to human *C* region polypeptides. Antibody secreted by transfected J558L cells expressing only the chimeric heavy chain and the endogenous J558L light chain did not bind *P*Cho (data not shown).

Further verification of the appropriate polypeptide folding of the mouse V_H and V_L domains attached to human *C* region polypeptide chains was done by analyses detecting the presence of idiotopes known to occur on the parental S107 *P*Cho-binding antibody molecule. Three monoclonal anti-idiotope antibodies—two recognizing idiotopes requiring the association of light and heavy *V* region domains (TC102.1.2 and T139.2) and the third (T156.1.1) recognizing an epitope present on the heavy chain *V* region domain (12)—were found to react with the mouse–human chimeric anti-*P*Cho antibodies (see Fig. 3). This is good evidence that the mouse S107 antigen-binding domains have folded into their intended structures.

The conformation of IgG heavy chain *C* regions depends

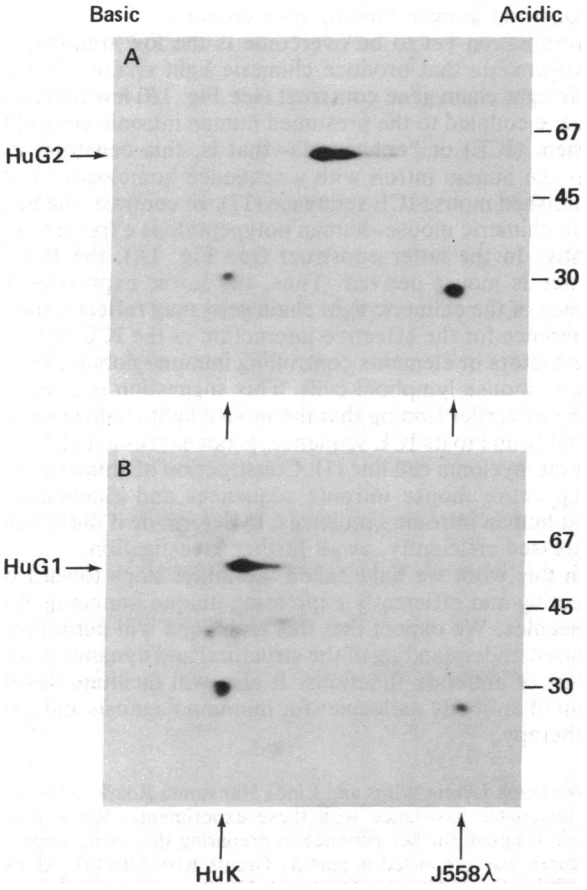

FIG. 2. Autoradiographs of two-dimensional NEPHGE gels of mouse–human chimeric IgG2 (κ) anti-PC antibody (*A*) and mouse–human chimeric IgG1 (κ) anti-PC antibody (*B*). Antibodies synthesized by transfected J558L cells were affinity purified with *P*Cho-Sepharose 4B. The electrophoretic mobility of the J558L λ chain had been determined previously (1). Size markers are shown in kDa.

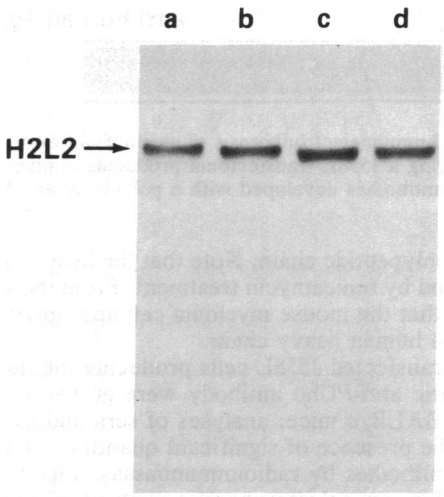

FIG. 3. Autoradiograph of nonreduced NaDodSO$_4$/PAGE analyses of *P*Cho-binding material from a chimeric IgG2 (κ) antibody-producing J558L cell line immunoprecipitated with *Staphylococcus aureus* protein A (lane a), monoclonal anti-human κ antibody (lane b), and anti-idiotope antibodies TC102.1.2 and T156.1.1 (lanes c and d).

on the presence of the asparagine-linked carbohydrate moeity in the CH_2 domain of the molecule (4). The loss of this carbohydrate chain affects profoundly the overall domain structure of this part of the immunoglobulin molecule. Concomitant with this structural change, the catabolism rate of the molecule is increased and biological effector functions such as complement-fixation are lost (14). Glycosylation of the mouse–human chimeric antibodies in mouse myeloma cells was deduced by determining the molecular masses of antibodies synthesized in the presence and absence of tunicamycin, an antibiotic inhibitor of asparagine-linked glycosylation. Fig. 4 is an autoradiogram of NaDodSO$_4$/PAGE analysis of glycosylated and nonglycosylated chimeric heavy and light chains produced in transfected mouse myeloma cells. Though we cannot be certain that in the absence of tunicamycin the appropriate asparagine residue in the CH_2 domain is glycosylated, clearly, the lower molecular mass of the heavy chain synthesized in the presence of tunicamycin is as expected if a single N-linked carbohydrate were absent

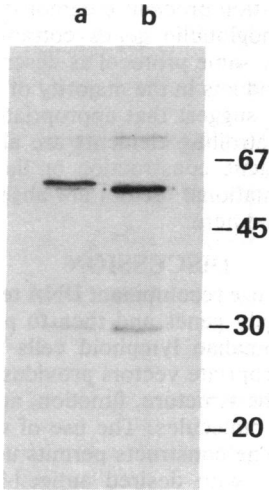

FIG. 4. Autoradiograph of reduced NaDodSO$_4$/PAGE analyses of chimeric IgG2 (κ) antibody synthesized in the presence (lane a) and absence (lane b) of tunicamycin. Size markers are shown in kDa.

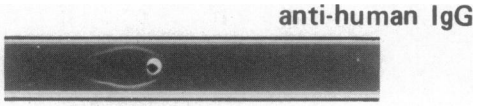

FIG. 5. Immunoelectrophoresis of ascitic fluid from a BALB/c mouse bearing a J558L transfectoma producing chimeric IgG2 (κ) anti-*P*Cho antibodies developed with a polyclonal anti-human IgG antiserum.

from the polypeptide chain. Note that the light chain band is not affected by tunicamycin treatment. From these data, we conclude that the mouse myeloma cell appropriately glycosylates the human heavy chain.

When transfected J558L cells producing the human IgG2 (κ) chimeric anti-*P*Cho antibody were grown as an ascitic tumor in BALB/c mice, analyses of sera and ascitic fluids showed the presence of significant quantities of anti-*P*Cho-binding antibodies by radioimmunoassay. Fig. 5 shows the results of immunoelectrophoretic analysis of ascitic fluids from these transfectoma-bearing mice. Polyclonal anti-human antiserum (a gift of E. A. Kabat, Columbia University, New York) was used to demonstrate the presence of human immunoglobulin. Based on our previous experience with mouse hybridoma-antibody production in mice, we conclude that the amounts of immunoglobulin visualized by this immunoelectrophoretic analysis are similar to the levels seen with lower-producing mouse hybridomas *in vivo*.

Transfectomas producing chimeric heavy and light chains were produced either by cotransfection of both genes using calcium phosphate precipitation or by sequential protoplast fusion using vectors with different drug markers. For protoplast fusion, the heavy chains were introduced first on a plasmid expressing the *Eco-gpt* gene. One heavy chain-producing cell line then was transfected by protoplast fusion with a plasmid containing the light chain gene and the neo marker. Transformants resistant to both mycophenolic acid and G418 were assayed for their production of anti-*P*Cho antibodies. Of 77 transformants analyzed, only 7 were positive for antibody by radioimmunoassay and only 2 of these were found to be producing significant quantities of κ light chain. In cotransfection experiments using the calcium phosphate precipitation protocol, the same phenomenon was observed. Analyses of a large number of independent transformants revealed that a minority (<10%) of the transfected cell lines produced both chimeric heavy and light chain polypeptides. Since our own unpublished data using the calcium phosphate precipitation procedure demonstrate that two intact mouse immunoglobulin genes cotransfected into the same cell, using the same protocol as described here, coexpress both gene products in the majority of transformed cell lines, these results suggest that appropriate transcriptional or translational controlling elements are absent in the chimeric light chain gene construction or that required transcriptional or translational factors are absent in the mouse cell lines transfected here.

DISCUSSION

The opportunity to use recombinant DNA techniques to construct novel antibody genes and then to produce antibody molecules in mammalian lymphoid cells transfected with these genes in appropriate vectors provides a new approach to understanding the structure, function, and immune properties of antibody molecules. The use of chimeric mouse–human antibody gene constructs permits us to study "near-human" antibodies with desired antigen-binding specificities. For clinical applications, human antibody molecules constructed by using recombinant DNA techniques should complement human–human hybridoma antibodies as useful immunotherapeutic and diagnostic reagents. The use of human the immunogenicity of antibodies used *in vivo*, relative to monoclonal mouse hybridoma antibodies currently being used (15). Further, since the same *V* region can be joined to any *C* region, it is possible to use the *C* region of an appropriate human heavy chain isotype that will exhibit desired biological effector functions.

The use of mammalian lymphoid cells to produce these antibodies rather than a prokaryotic expression system assures that any post-translational modifications of the antibody molecules as they are synthesized, processed, folded, and assembled in the eukaryotic endoplasmic reticulum are carried out correctly. For example, the immunoglobulins encoded by the transfected mouse–human chimeric immunoglobulin genes in both mouse recipient cells were glycosylated, bound antigen, and presumably would be treated as normal immunoglobulins if injected into humans. This obviates the need for any post-synthetic *in vitro* modification or assembly that is required with prokaryotic synthesis of immunoglobulin polypeptides to produce functional antibody molecules (16). Furthermore, we have shown that transfectomas produce useable amounts of human antibodies either in tissue culture or in mice.

It is now feasible to shuffle exons or carry out other kinds of directed mutagenesis to explore the human antibody molecules *C* region structures required for carrying out diverse antibody functions. It is conceivable that an altered antibody molecule more effective in specific functions than naturally occurring antibody molecule can be created. In a similar manner, it should be possible to modify *V* regions to alter their interactions with antigen. Even more exciting would be to construct antigen-binding sites *de novo*.

A limitation yet to be overcome is the low frequency of transformants that produce chimeric light chains. The chimeric light chain gene construct (see Fig. 1*B*) has the mouse V_κ gene coupled to the presumed human intronic controlling element (ICE) or "enhancer"—that is, this construct contains the human intron with a sequence homologous to the established mouse ICE sequence (17). In contrast, the heavy chain chimeric mouse–human polypeptide is expressed efficiently. In the latter construct (see Fig. 1*A*), the ICE sequence is mouse-derived. Thus, the lower expression frequency of the chimeric light chain gene may reflect a species preference for the effective interaction of the ICE sequence with factors or elements controlling immunoglobulin expression in mouse lymphoid cells. This suggestion is consistent with our earlier finding that the mouse light chain gene, presumably due to its ICE sequence, is not expressed efficiently in a rat myeloma cell line (1). Construction of chimeric genes using entire mouse intronic sequences and elimination of most human intronic sequences, to determine if these will be expressed efficiently, await further investigation.

In this work we have taken the initial steps toward constructing and efficiently expressing unique immunoglobulin molecules. We expect that this technique will permit an increased understanding of the structural and dynamic requirements of antibody functions. It also will facilitate development of antibody molecules for immunodiagnosis and immunotherapy.

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