Transfectomas Provide Novel Chimeric Antibodies

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Summary. Methods have been developed to transfect immunoglobulin genes into lymphoid cells. The transfected genes are faithfully expressed, and assembly can occur both between the transfected and endogenous chains and between two transfected chains. Gene transfection can be used to reconstitute immunoglobulin molecules and to produce novel immunoglobulin molecules. These novel molecules can represent unique combinations of heavy and light chains; alternatively, by means of recombinant DNA technology, genes can be assembled in vitro, transfected, and expressed. The end products of such manipulations include chimeric molecules with variable regions joined to different isotypic constant regions; this is possible both within and between species. It is also possible to synthesize altered immunoglobulin molecules, as well as molecules having immunoglobulin sequences fused with nonimmunoglobulin sequences (for example, enzyme sequences).

Nearly 100 years ago it was shown that the antibodies circulating in the serum provided the basis for the immune response. With the development of serum electrophoresis it was possible to demonstrate that these antibodies were protein molecules (*1*). Many years of research have revealed that antibodies are responsible for specific protection against bacterial and viral diseases and are involved in normal and disease-related immune reactions, including inflammation, autoimmunity, graft rejection, and idiotype-mediated network regulation. Antibodies have also proven to be invaluable, exquisitely sensitive reagents for the location, identification, and quantification of antigens in many different assay systems.

The advent of hybridoma technology in 1975 made it possible to obtain antibodies of a defined specificity in large quantities (*2*). However, limitations persisted, and it was not always possible to generate antibodies with the precise specificity desired or with the appropriate combination of specificity and effector functions. In addition, hybridomas cannot be produced with equal ease from all species; in particular, human hybridoma antibodies have been difficult to obtain. Thus, an exciting recent advance has been the development of transfectomas, in which a combination of recombinant DNA techniques and gene transfection can be used to create novel, chimeric immunoglobulin molecules.

Structural Basis of Antibody Function

Two functions are characteristic of every antibody molecule: (i) specific binding to an antigenic determinant, and (ii) participation in effector functions, such as binding and activation of complement, stimulation of phagocytosis by macrophages, and triggering of granule release by mast cells. The specific binding of antigen by antibodies is determined by the structure of the variable regions of both heavy (V_H) and light (V_L) chains (see Fig. 1). The effector functions are determined by the structure of the constant region (C) of the heavy chains. Immunoglobulins (Ig's) with different constant regions—that is, Ig's of differing isotypes (in the human IgM, IgD, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2, and IgE)—therefore exhibit different biologic properties. Antibodies are glycoproteins, and the presence of carbohydrate on the antibody molecule is essential for some of the effector functions of antibodies (*3*). Cleavage with the enzyme papain splits the Ig molecule into an Fab region that can bind antigen and into an Fc region containing the effector functions (Fig. 1).

Both the heavy and light chains are encoded by multiple DNA segments. A functional Ig gene is generated only after somatic rearrangement of distinct DNA segments (*4*). In the functional Ig gene, intervening sequences separate the hydrophobic leader sequence from the variable region and also the variable-region gene segment from the constant region; in addition, intervening sequences separate the different domains of the constant region in heavy chains so that each functional region of the heavy chain constant region is on a separate exon (Fig. 1).

In the past, structural and functional studies of Ig molecules were complicated by the heterogeneity of the molecules. Because antibodies present in an antiserum are a mixture of specificities, antisera of defined specificities were difficult to obtain in large quantities. These problems were solved to a certain degree when it was realized that the disease multiple myeloma represents a monoclonal proliferation of plasma cells and that large quantities of homogeneous antibodies could be obtained. The majority of what is known about the structure of antibody molecules and the organization of Ig genes has come from the study of these myeloma proteins and cells. Unfortunately, only a few of the myeloma proteins can be demonstrated to bind known antigens, so their usefulness as specific reagents is severely limited.

Monoclonal antibodies of defined specificity became available when Köhler and Milstein demonstrated that cultured mouse myeloma cells could be fused to spleen cells from immunized mice (*2*). The hybrid cells (hybridomas) grow continuously in culture (property acquired from the myeloma cell) and continue to produce in large quantities the Ig that had been synthesized by the spleen cell partner. These antibody molecules are monoclonal; hence, they have an advantage over classical antisera in that their combining sites are homogeneous. However, the fact that the Ig produced by one hybridoma is of only one isotype can be a problem if the isotype expressed does not have the effector function, such as complement fixation or binding of staphylococcal protein A, required for a certain assay.

The large quantities of chemically defined antibodies produced by hybridomas have been invaluable tools for immunologists, but they do have certain limitations. Although hybridoma proteins that bind defined antigen can be identified, the affinity of the binding cannot be determined by the researcher.

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Furthermore, the isotype of the antibody is determined by the constant region gene being used by the normal cell at the time of fusion.

An additional problem associated with hybridomas is their species limitations. While it is relatively easy to produce mouse or rat monoclonal antibodies, it is difficult to produce human monoclonal antibodies, which would be desirable for certain studies and in vivo use. Many human hybridomas produce only limited quantities of Ig and are unstable in their production.

The isolation of somatic mutants of hybridoma cells has provided investigators with additional capabilities. Cell lines with both decreased and increased affinity for antigen can be isolated (*5*). In addition, it is possible to isolate somatic mutants with alterations in their Fc regions (*6*). These mutations may be either structural changes in the Fc region or may represent isotype switch mutants. Switch variants have permitted an assessment of the contribution of the hinge region to the segmental flexibility of Ig molecules (*7*).

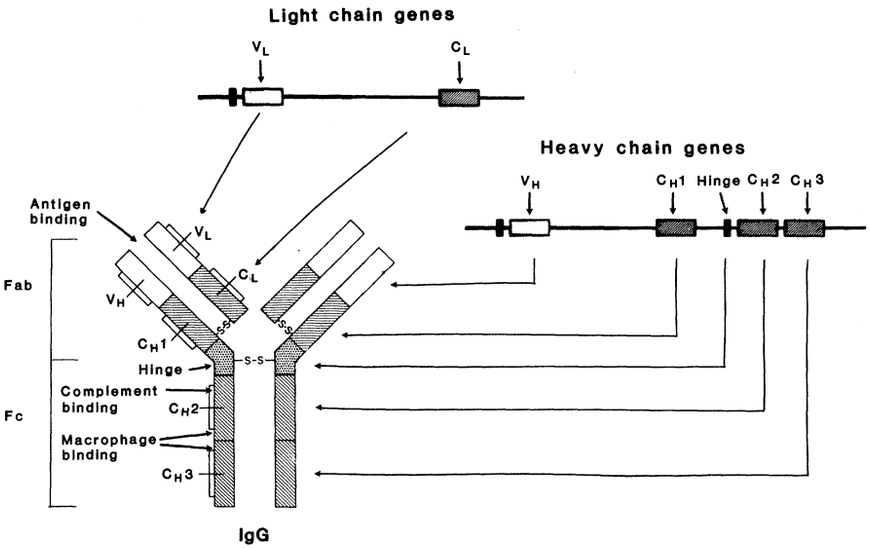

Fig. 1. Structure of an Ig molecule and the genes that encode it. The regions of the molecule that participate in antigen binding (Fab) or different effector functions (Fc) are indicated. Arrows indicate the correspondence between the DNA segments and the different domains of the Ig polypeptide chain that they encode. The hydrophobic leader sequence of both heavy and light chains is removed immediately after synthesis and is not present in the mature immunoglobulin molecule.

Ig Gene Transfection into Lymphoid Cells

Gene transfection provides a method for making novel Ig molecules (Fig. 2). Ig genes, either wild-type or altered in vitro, can be transfected and expressed (*8, 9*). The transfected cells (transfectomas) grow continuously in culture and produce the Ig specified by the transfected gene. This approach has the advantage over the isolation of mutants that alteration can be predetermined and does not depend on the chance occurrence of the desired changes.

The expression of transfected genes can be studied transiently (a few days after transfection) or stable transfectants in which the transfected gene has integrated into the chromosome can be isolated. If the objective is to produce a new protein, stable transfectants need to be isolated to provide a continuous source of the protein. Because only a small percentage (10^{-3} to 10^{-6}) of the cells exposed to foreign DNA go on to become stably transformed, selective techniques are required that permit the isolation of rare, stably transformed cells from among the many nontransformed cells. Initial experiments used the thymidine kinase gene from herpes simplex virus as the selective marker (*10*). However, this required that the recipient cells be deficient in endogenous thymidine kinase (TK^-); consequently, only a few available cell lines lacking thymidine kinase could be used as recipients.

To overcome these limitations, vectors with dominant selectable markers have been developed (*11–13*). Dominant-acting genetic markers produce a selectable change in the phenotype of normal cells; such markers have been produced by placing bacterial genes within mammalian transcription units. The most commonly used vectors have been the pSV2 vectors (*11*), in which the selectable marker is placed under the control of the SV40 early promoter; SV40 sequences provide splice signals and a polyadenylation site.

Two selectable bacterial genes have been used: (i) the xanthine-guanine phosphoribosyltransferase gene (*gpt*) (*12*), and (ii) the phosphotransferase gene from Tn5 (designated *neo*) (*13*). Selection with *gpt* is based on the fact that the enzyme encoded by this gene can use xanthine as a substrate for purine nucleotide synthesis, whereas the analogous endogenous enzyme cannot. Thus, for cells provided with xanthine and in which the conversion of inosine monophosphate to xanthine monophosphate is blocked by mycophenolic acid (*14*), only cells expressing the bacterial gene can survive. The product of the *neo* gene inactivates the antibiotic G418 (*15, 16*), which interferes with the function of 80*S* ribosomes and blocks protein synthesis in eukaryotic cells. The two selection procedures depend on two entirely different mechanisms; therefore, they can be used simultaneously to select for the expression of genes introduced on two different DNA segments. Alternatively, they can be used to select for the expression of different genes introduced sequentially into cells.

The recipient cell type of choice for the production of Ig molecules would appear to be myeloma cells. Myelomas represent malignancies of plasma cells, and they are capable of producing large quantities of Ig. Expression of transfected heavy chain genes by myelomas approaches the level seen for the endogenous myeloma protein (*17, 18*). Expression of light chain genes after transfection has been more of a problem, and frequently it is only 5 to 10 percent of that seen in myeloma cells expressing the same gene (*19*). However, it has been possible to identify transfectants in which the synthesis of both the light and heavy chain of the functional Ig molecule has been directed by a transfected gene (*20–22*).

Several methods exist for introducing DNA into eukaryotic cells. Calcium phosphate precipitation is routinely used to introduce DNA into many cell types (*10, 23, 24*). However, this technique results in a low frequency of recovery of transfectants from myeloma cells (*9*). A more efficient way of introducing DNA into lymphoid cells is by protoplast fusion (*9, 24*). In this method, lysozyme is used to remove the bacterial cell walls from *Escherichia coli* bearing the plasmids of interest (*9, 24*), and the resulting spheroplasts are fused with myeloma cells by means of polyethylene glycol. After protoplast fusion, stable transfectants have been isolated at frequencies

ranging from 10^{-4} to 10^{-3} (*9, 17, 20*). Recent results have suggested that electroporation may also be an efficient method for introducing DNA into lymphoid cells (*25*). For electroporation, a high-voltage pulse is applied to cells in suspension in the presence of DNA. Stable transfectants have been isolated from many different cell types at frequencies as high as 3×10^{-4}.

Variations in transfectability have been observed among the different myeloma cell lines and among different clones of the same myeloma cell line. The cause of the variations in unknown. The myeloma J558L, which produces λ light chains and no heavy chains, is among the best recipient cell lines. With the appropriate vectors, it can be transfected with efficiencies approaching 10^{-3} (*9, 17, 26, 27*). However, J558L has the disadvantage that it continues to synthesize its own λ light chain; it has been impossible to isolate nonproducing variants of J558L. Although some nonproducing myelomas, such as SP2/0, have been transfected, the transfection frequencies are 10^{-1} to 10^{-2} of that seen with J558L.

Expression of Transfected Genes

It is necessary to prove that the proteins synthesized after gene transfection are the same as those synthesized in myeloma cell lines. When the MOPC-41 κ light chain was introduced into a cell line that had been transformed with Abelson murine leukemia virus, the cells directed the synthesis of a κ chain that could assemble with the endogenous γ_{2b} heavy chain to produce molecules containing two heavy and two light chains (H_2L_2) (*8*). The light chain produced after the transfection of the S107A κ chain was identical, by two-dimensional gel analysis, to the S107A myeloma κ chain (*9*). The S107A light chain is not secreted by light chain–producing variants of this myeloma; a transfected S107A light chain was also not secreted when introduced into a myeloma that did not produce any heavy chain. However, when the S107A κ chain was introduced into a hybridoma cell line that synthesized a heavy chain, it assembled with the endogenous heavy chain to produce H_2L_2 molecules; these H_2L_2 molecules were secreted.

By means of genes isolated from a trinitrophenyl (TNP)-binding hybridoma, the faithful expression of transfected genes with the retention of the original antigen-binding specificity was seen (*20, 28*). Transfection of the light chain from this hybridoma into a cell line that synthesized the TNP-specific heavy chain, but that had lost the ability to synthesize the TNP-specific light chain, resulted in the synthesis of a complete H_2L_2 molecule with the ability to bind antigen. This TNP-specific IgM was secreted by the transfected cells, and the hemagglutination titer of antibody to TNP (anti-TNP) in some transformants was comparable to that of the parental anti-TNP–producing hybridoma. Conversely, by transferring a μ heavy chain gene from an anti-TNP–specific myeloma into a light chain–producing variant of that myeloma, it was also possible to express a pentameric IgM molecule with anti-TNP activity. Simultaneous transfer of specific light and heavy chains into a nonproducing myeloma also resulted in the production of pentameric, antigen-binding antibody, although in this case the amount of antibody synthesized was less than that seen in the parental myeloma.

Gene transfection can be used to identify tissue-specific regulatory elements.

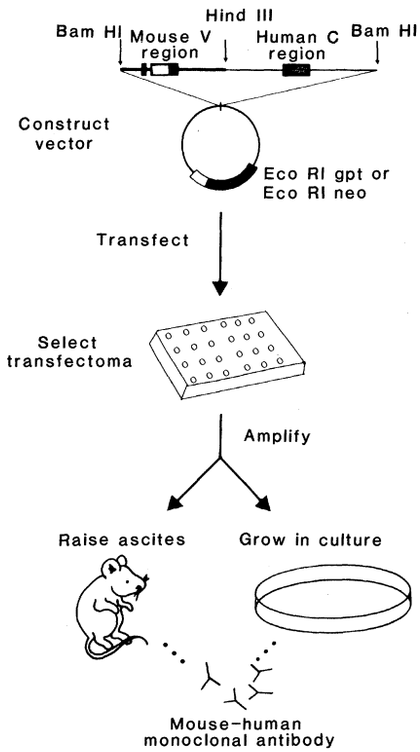

Fig. 2. Steps in production of chimeric Ig molecules. First, a chimeric gene containing the sequences of interest is ligated into an expression vector containing a selectable marker. Then, this vector is transfected into the appropriate recipient cell line, and stable transfectants synthesizing the genes of interest are identified; if vectors are used with independent selectable markers, transfectants synthesizing more than one novel chain can be isolated. Finally, the chimeric proteins are isolated either from tissue culture or from the ascitic fluid of tumor-bearing mice.

A segment of DNA located between J_4 and the switch region in the major intervening sequence of a heavy chain gene is necessary for the expression of heavy chain genes transfected in lymphoid cells of the B lineage (*17*); this region does not influence the expression of Ig genes transfected into L cells (*29*). The location of this DNA segment 5′ of the switch site guarantees that the segment will remain associated with the expressed variable region gene during isotype switching and is consistent with the segment having a regulatory role. However, cell lines have been reported in which deletion of this segment does not abolish heavy chain synthesis (*30*). Therefore, a question remains as to relationship between the sequences needed for the expression of transfected genes and those needed for the expression of endogenous genes.

Controlling regions have been identified in the major intervening sequence of κ light chains. A mouse sequence lying approximately 600 base pairs 5′ of C_κ enhanced production of κ chain genes transfected into lymphoid cells (*19, 31*). Like the heavy chain controlling region, it does not function in nonlymphoid cells. This sequence corresponds to a DNA segment (*32*) that is highly conserved among rabbit, mouse, and man and that becomes sensitive to deoxyribonuclease when Ig is expressed (*33*). In addition, a second DNA segment from the 5′ half of the κ intervening sequence has been identified that facilitates expression of κ genes transfected into mouse myeloma but not into hamster lymphoma cells (*19, 34*).

Production of Novel Ig Molecules by Gene Transfection

With the demonstration that transfected Ig genes faithfully exhibited the expected properties, it became possible to produce novel Ig molecules. These novel Ig's can be divided into two categories: (i) those synthesizing wild-type Ig chains but in a combination not normally expressed and (ii) those synthesizing molecules with a novel gene structure that has been produced by recombinant DNA techniques (Table 1).

Novel combinations of wild-type heavy and light chains. In vitro reassembly of Ig heavy and light chain polypeptides has been used to create novel heavy-light chain combinations and to study chain interactions (*35–37*). This method, however, requires that the original interchain disulfide bonds in the Ig be broken and that heavy and light chains be separated in the presence of denatur-

ing agents. As a result, only a small percentage of the chains renature to produce functional molecules (*36*). On the other hand, chain assembly between transfected gene products occurs within the cell by normal pathways of assembly. Because the assembled molecules have never been subjected to a denaturing step, their structure and function should more accurately reflect those of native molecules.

Experiments either with cell lines transformed by Abelson murine leukemia virus or with hybridomas have indicated that assembly can occur between the transfected and the endogenous Ig chains (Fig. 3) (*8, 9*), and this has been found to be generally true. When the γ_{2b} heavy chain of the myeloma MPC-11 was transfected into the λ myeloma J558, it was expressed (Fig. 3, lane 3), assembled with the endogenous λ chain, and secreted as H_2L_2 molecules (Fig. 3, lane 4). These novel molecules, in which the MPC-11 heavy chains are associated with λ light chains, have been useful in exploring the relative contribution of V_H and V_L to idiotypic determinants (*38*).

Novel combinations of variable and constant regions. By means of standard recombinant DNA techniques, it is possible to attach any variable region to any heavy chain constant region. The existence of the large intervening sequence between the two regions (see Fig. 1) makes such joining easier. Because the reading frame does not have to be maintained and the intervening sequence will be removed by splicing, many different joints will lead to functional molecules. This approach can be used to produce isotype switch variants of mouse hybridoma proteins.

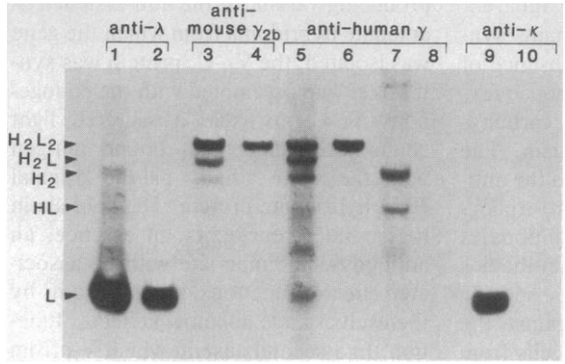

Fig. 3. Synthesis of Ig chains in transfectomas. Cells were labeled with [^{14}C]valine, [^{14}C]threonine, and [^{14}C]leucine for 3 hours, and cytoplasmic lysates and secreted material were immunoprecipitated with the antibodies shown at the top of the figure. (Lanes 1 and 2) J558L; (lanes 3 and 4) J558L containing a transfected MPC-11 γ_{2b} heavy chain; (lanes 5 and 6) J558L containing a chimeric mouse-human heavy chain; (lanes 7 and 8) nonproducing myeloma cells containing a chimeric mouse-human heavy chain; and (lanes 9 and 10) V_HC_κ in light chain–producing variant of anti-phosphocholine myeloma S107. Lanes 1, 3, 5, 7, and 9 represent cytoplasmic extracts; lanes 2, 4, 6, 8, and 10 represent secreted material.

Another family of molecules can be created in which the variable regions from the heavy and light chains of a mouse myeloma are joined to human constant regions (Fig. 2). These molecules have the antigen-binding specificity of the mouse hybridoma, but they should exhibit the effector function associated with the human constant regions and should be less antigenic in humans than are totally mouse antibodies.

Initially, the variable region from the heavy chain of the anti-phosphocholine myeloma S107 was joined to either human γ_1 or γ_2 heavy chain (*21*). After transfection into J558L, the chimeric gene was expressed (Fig. 3, lane 5), the chimeric heavy chain was assembled with the endogenous λ chain, and H_2L_2 molecules were secreted. The chimeric heavy chain was also expressed by myeloma cells not synthesizing any light chains (nonproducing) (Fig. 3, lane 7); however, these heavy chains were not secreted (Fig. 3, lane 8).

Subsequently, the antigen-specific light chain of S107 was joined to human C_κ and ligated into the pSV2neo vector, and transfectants synthesizing both chimeric heavy and light chains were identified (*21*). The chimeric heavy and light chains of the expected molecular weight were assembled and secreted as H_2L_2

Table 1. Creation of novel molecules by gene transfection.

Genes used for transfection	Objective	Potential uses
	Wild-type Ig chains	
Wild-type heavy or light chains (or both)	To create new heavy and light chain combinations, both within a species and between species	Explore the nature of heavy and light chain interactions
		Define the relative contributions of each chain to idiotype expression and antigen-binding specificity
		Determine if all chain combinations and thus combinational diversity are possible
	Novel Ig chains	
V_H attached to new C_H; V_L attached to new C_L	To create chains with the same variable region associated with a new isotype	Produce isotype switch variants within one species
		Create cross-species chimerics, with a variable region from one species and a constant region from another
V_H attached to C_L; V_L attached to C_H	To create chains with a variable region attached to an isotype with which it is never associated in vivo	Evaluate contributions of constant regions to functions
		Create molecules with altered effector functions
V_H or V_L attached to a non-Ig sequence	To create fusion proteins	Attach antibody specificity to enzymes for use in assays
		Isolate non-Ig proteins by antigen columns
		Specifically deliver toxic agents

molecules. Comparison of the apparent molecular weights of heavy chains synthesized in the presence and absence of tunicamycin showed that the mouse myeloma cell attaches *N*-linked carbohydrate to the human heavy chain. The chimeric H_2L_2 molecules bound the antigen phosphocholine and were recognized both by monoclonal antibodies specific for human κ chain antibodies and by monoclonal antibodies specific for the idiotype prepared against the mouse myeloma protein. The cells from the transfectoma produced an ascites after being injected into a mouse, and the chimeric proteins were found to be present in the ascitic fluid.

Chimeric human-mouse molecules have also been produced in which the variable regions of the heavy and light chains from an anti-TNP mouse myeloma were linked to human μ and κ genes, respectively, and were transfected into myeloma cells (*22*). The chimeric genes were expressed and assembled such that pentameric IgM was present in the secretions; however, both the chimeric μ and κ chains migrated more slowly than was expected on gels containing sodium dodecyl sulfate. There was no significant difference in affinity for TNP between the mouse and the chimeric IgM's. The chimeric IgM, however, was about one-fourth as effective as the mouse IgM in hemolysis of TNP-coupled sheep red blood cells and showed a displaced binding curve in hapten inhibition assays. The molecular basis for these apparent binding differences remains unclear.

Another chimeric molecule has been produced in which a heavy chain with the variable region of a mouse antibody to 4-hydroxy-3-nitro-phenacetyl (NP) was joined to human ε heavy chain (*27*). This fused segment was transfected into the J558L cell line, which synthesizes a mouse λ light chain identical to that used by mouse antibody to NP. The J558L λ chain assembled with the chimeric heavy chain to produce H_2L_2 molecules, which were secreted and, like human ε, were heavily glycosylated. The chimeric IgE bound NP efficiently and, when the chimeric antibodies were bound to the appropriate receptors on human basophils, antigen was able to stimulate a dose-dependent release of histamine.

Variable regions can also be placed on constant regions with which they would not normally be associated. When the V_H from an anti-azophenylarsonate hybridoma was joined to mouse C_κ and introduced into a mouse myeloma cell, a 25,000-dalton chimeric protein was produced (*39*). When the chimeric V_H-C_κ gene was transfected into a light chain–producing variant of the anti-azophenylarsonate hybridoma from which the gene was isolated, the V_H-C_κ protein was synthesized and assembled with the endogenous V_L-C_κ to yield a secreted light chain heterodimer that bound antigen with the same affinity as the original H_2L_2 hybridoma protein. The light chain heterodimer represents, in essence, an antibody-combining site with no associated effector functions, as light chains by themselves have no known effector function. In a second case in which V_H from the S107 myeloma was joined to Cκ and introduced in a light chain–producing variant of S107, a 25,000-dalton chimeric protein was produced, but heterodimers were not secreted (Fig. 3, lanes 9 and 10).

The V_H from a human myeloma can be joined to mouse C_κ (*40*). Although this human-mouse chimeric protein was produced, it did not assemble with the endogenous λ light chain of the recipient mouse myeloma and remained cytoplasmic. These experiments demonstrated that the human heavy chain promoter can function in mouse myelomas. Additional studies have shown that the human light chain promoter also can function in mouse myeloma cells (*25*). It is thus possible to consider using gene transfection techniques for gene rescue; that is, genes could be cloned from human myelomas or lymphoblastoid lines where they are expressed at low levels and then introduced by gene transfection into mouse myeloma lines. These initial studies suggest that such transfected genes will effectively function to produce high levels of protein.

Gene transfection has also been used to create cell lines synthesizing $F(ab')_2$-like antibody (*26*). [$F(ab')_2$ is two Fab's joined by disulfide bonds (see Fig. 1)]. A chimeric gene was constructed in which the variable region of a mouse NP-binding heavy chain was joined to the C_H1 and hinge regions of mouse γ_{2b}. The fifth exon from the δ heavy chain provided translation termination and polyadenylation signals at the end of the heavy chain. This chimeric gene was transfected into λ_1-producing J558L cells; the λ_1 light chain associated with the NP-specific heavy chains to produce an antigen-binding protein that could be isolated from culture supernatants of transfected cells. The protein had a molecular weight of approximately 110,000 daltons and, on reduction, yielded one band that comigrated with λ light chain and several other bands of higher molecular weight; the predominant species was approximately 31,000 daltons in size. It thus appeared that an $F(ab')_2$-like antibody to NP was synthesized and secreted in large quantities.

Chimeric molecules with Ig sequences joined to non-Ig sequences. Finally, chimeric molecules can be produced in which Ig and non-Ig sequences are joined. The gene for *Staphylococcus aureus* nuclease was inserted into the C_H2 exon of a mouse γ_2 heavy chain specific for NP, and the construction was transfected into J558L cells (*26*). The chimeric heavy chain was produced and assembled with the λ light chain to form an NP-binding protein. Molecules of the appropriate size to be H_2L_2 were isolated from the secretions; these molecules bound the antigen NP and also had nuclease activity that, like the activity of authentic *S. aureus* nuclease, was dependent on Ca^{2+} but not Mg^{2+} ions. On a molar basis, the catalytic activity of the constructed nuclease was about 10 percent of that of authentic *S. aureus* nuclease. Similarly, in another study, the C_H2 and C_H3 domains of the heavy chain were replaced with the third exon of c-*myc*. After transfection into J558L cells, a secreted protein was produced that retained its ability to bind antigen and that was recognized by a monoclonal antibody to c-*myc*. These molecules show the feasibility of making unique combinations of antibody and protein functions that are secreted in large quantities by myeloma cells and retain their ability to bind antigen; hence, they can be rapidly purified on antigen columns.

Expression of Ig Genes in Nonlymphoid Cells

Expression of Ig genes and synthesis of Ig molecules in bacteria provide an alternative to expression in lymphoid cells. Bacteria have been used to make fragments of ε heavy chain that were at least partially biologically active in that they bound to the IgE receptor on basophils (*41–43*). However, heavy chains, light chains, and light chain fragments made in bacteria became insoluble components of inclusion bodies (*42–44*) rather than functionally intact molecules. Assembly did not occur even when efforts were made to promote heavy and light chain assembly by including them within the same bacterium on two different plasmids. Antigen-binding Ig molecules that bound antigen could be produced only by in vitro assembly and at very low yields (*44*). In addition, bacteria have the inherent limitation that they do not glycosylate proteins; therefore, any biologic function of Ig's that depends on carbohydrate would be missing.

Yeast can glycosylate proteins and hence may be preferred over bacteria for Ig expression. Recently, the synthesis in yeast of both λ light chains and μ heavy chains has been reported (*45*); a portion of the μ chains was *N*-glycosylated. Both the λ and μ chains were secreted, and, when μ and λ chains were expressed together, some assembly into antibody molecules that bound antigen occurred. However, the specific activity of antibody molecules isolated from a soluble extract of yeast cells was only about 0.5 percent, indicating that the efficiency of heavy and light chain assembly into functional molecules was low.

Future Perspectives and Applications

The initial experiments demonstrated the feasibility of producing novel Ig molecules by gene transfection. Myeloma cells appear to be the best recipient for the Ig genes because the transfected Ig genes are faithfully transcribed, translated, and glycosylated in them. The antibody components are synthesized in large amounts and can be assembled either with endogenous Ig chains or with other transfected chains to produce functional molecules. Nonlymphoid cells synthesize only small amounts of Ig, and both bacteria and yeast have proved to be inefficient in assembling functional Ig molecules.

Chimeric Ig molecules should provide a new family of reagents of wide potential application. Changing the Fc portion of the Ig can alter its ability to bind staphylococcal protein A, to be multivalent, or to fix complement and, therefore, would affect the usefulness of the Ig for many in vitro assays. Molecules with increased or decreased binding affinity can be useful in creating detection assays that have different levels of sensitivity and stringency.

The ability to produce antibody-protein chimeras permits the construction of a molecule with the Fab of an Ig molecule covalently bound to a non-Ig protein. When the Fab is bound to an enzyme, the binding of the Fab to its antigen can be detected by addition of the appropriate substrate. If the Fab is bound to a toxin, it can potentially be used as a specific cytotoxic agent. In addition, chimeric Ig molecules can function to facilitate protein isolation in that the non-Ig protein attached to a functional antibody-combining site can be purified on an antigen column.

In vitro mutagenesis may provide an additional way to generate molecules of altered function. It should be possible to generate molecules lacking only one specific effector function, with the remainder of the molecule intact; to eliminate or generate glycosylation sites; and through small changes in the variable region, to increase or decrease affinity for antigen. In turn, analysis of the exact structure of the mutated region is expected to reveal those areas of the Ig molecules required for effector function or antigen binding.

Human antibodies would clearly be optimal for use in man, but it has been difficult to produce human hybridoma that synthesize large quantities of antibodies with the desired specificities. Mouse antibodies have been used in trial studies for the treatment of certain human diseases. However, on continued use, they frequently elicit an immune response that renders them noneffective (*46*). Chimeric molecules may help solve this problem because molecules in which only the variable region is of nonhuman origin should be much less antigenic than completely foreign molecules. Chimeric molecules may prove useful in antibody-mediated cancer therapy and in the treatment of certain autoimmune diseases (*47*).

Even in the diagnosis or treatment of diseases in which chronic exposure to the treatment agent is not necessary (resulting in fewer anticipated problems with the agent's antigenicity), the use of chimeric molecules would appear to be preferable to the use of totally foreign Ig. Such chimeric antibodies are potentially useful, for example, for the treatment of immunosuppressed individuals who are subject to fatal viremias and of nonimmunized individuals exposed to tetanus who would be treated with horse antitoxin. Use of chimeric molecules also provides a choice of Ig's with the desired effector functions; alternatively, if no effector function is wanted, use of light chain heterodimers of IgA or Fab-type molecules should permit antigen clearing without complement activation. The application of these molecules to many clinical problems should be quite rewarding.

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