High-level production of a functional immunoglobulin heterodimer in a baculovirus expression system

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Communicated by Edwin D. Kilbourne, March 7, 1990 (received for review January 8, 1990)

ABSTRACT A murine immunoglobulin heterodimer has been expressed in a baculovirus expression system. This was achieved by using both double infection of insect cells with separate heavy- and light-chain-expressing viruses and infection with a double-recombinant virus containing both the immunoglobulin heavy- and light-chain cDNAs. In both cases, the polypeptide chains were correctly processed, glycosylated, and assembled into normal H₂L₂ (H = heavy, L = light) immunoglobulin monomers. These molecules bound antigen and expressed both polyclonal idiotype and monoclonal idiotopes. Furthermore, the transfer vectors described have been modified to contain the F1 origin of replication for the production of single-stranded DNA, which facilitates site-specific mutations of either the polyhedrin promoter or the inserted foreign gene. Use of this system should significantly advance the analysis of the structural bases for both idiotype expression and antigen binding by immunoglobulin. More importantly, it provides a generic method for the high-level expression of antibodies of diverse interest.

Approaches to the study of antibody structure/function have gone through many stages, especially with respect to the generation of suitable materials for study. Initially, polyclonal immunoglobulins, mixed cryoglobulins, or immunoglobulins isolated from patients with plasma cell tumors were studied (1–3). The powerful tool of hybridoma production provided high numbers of monospecific clones (4). However, despite the ability to create hybridomas, significant limitations on our ability to produce certain antibodies still exist. It is often desirable to have a means to produce antibodies with a precise amino acid sequence based solely on theoretical considerations and not simply those molecules that the immune system will produce and can be subsequently immortalized in a fusion.

To this end, *in vitro* systems for the expression of recombinant antibodies have been examined for their ability to produce functional antibodies. Transfection of eukaryotic expression vectors into immortalized B cells has been the most successful of the *in vitro* systems in terms of the reliable production of normal immunoglobulins (5–7). The amount of antibody produced in such systems is quite small, however, and it is difficult to establish stable transfectant cell lines. Efforts to express entire immunoglobulins in *Escherichia coli* have been unsuccessful. One group has succeeded in expressing an Fv fragment (8), whereas another has developed single-chain antigen-binding molecules (9). Although each of these molecules will bind antigen, neither will express the effector functions associated with the constant region domains or be suitable models for the study of the three-dimensional structure of normal antibody molecules.

To express intact immunoglobulins at a high level, we have taken advantage of a baculovirus-based expression system.

Baculovirus expression of foreign genes has been applied to the study of a wide variety of proteins from various organisms (10–12). Expression of proteins in this system has several advantages over either prokaryotic or transfection-based eukaryotic expression systems. First, bacteria do not correctly process and glycosylate eukaryotic proteins, whereas insect cells have been shown to do both (13, 14). Also, baculovirus expression of foreign protein has been shown to constitute as much as 50–75% of total cellular protein late in viral infection, an amount significantly greater than that produced in a transfected eukaryotic cell.

We report here the successful production of a functional murine antibody molecule (γ_1 , κ) in insect cells. Several approaches to baculovirus transfer vector construction are also reported, including the use of a phagemid vector and a double-recombinant, heterodimer-producing transfer vector.

MATERIALS AND METHODS

Viruses and Cells. *Autographa californica* nuclear polyhedrosis virus (AcNPV) and recombinant viral stocks were produced and assayed in *Spodoptera frugiperda* (Sf9) cells grown in TNM-FH medium/10% (vol/vol) fetal calf serum plus antibiotics essentially as described by Summers and Smith (15).

Construction of Baculovirus Transfer Vectors. The transfer vectors used in this study are diagrammed in Fig. 1. The 93G7 heavy-chain cDNA (γ_1) and 91A3 rearranged light-chain gene (κ) were cloned previously (16, 17). The baculovirus transfer vectors used in this study are based on pAc360 (13). pH γ_1 360E was derived from pAc360 in several steps: the F1 origin of replication was cloned into the single *EcoRI* site of pAc360, followed by the creation of an *Nco* I site at the translation initiation site for polyhedrin, and finally, the 93G7 heavy-chain cDNA was inserted into this unique *Nco* I site. The 91A3 κ gene had its introns removed by oligonucleotide-directed deletion mutagenesis, and this “cDNA” was cloned into the *Nco* I site as described above for the heavy chain. The vector pH γ_1 κ -360 contains a dicistronic coding segment; the 93G7 heavy chain precedes the 91A3 light chain. Finally, the construct pH γ_1 κ -Inv contains two complete transcription units in opposite orientations. The 3.9-kilobase *Sal* I fragment from pH κ -360 was ligated to pH γ_1 -360E cut with *EcoRV*. A clone from this ligation was selected that had the κ and γ coding units in opposite orientations. The coding and regulatory regions of all the clones described above were sequenced to verify that no unintentional mutations were introduced during their generation.

Transfection and Isolation of Recombinant Viruses. Cesium chloride gradient-purified plasmid DNA (2–20 μ g) was co-transfected into Sf9 cells with total cellular DNA from virally

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Abbreviations: Ars, *p*-azophenylarsenate; CRI, cross-reactive idiotype; CRI_A, cross-reactive idiotype of the V_HArs associated molecules of A strain mice; AcNPV, *Autographa californica* nuclear polyhedrosis virus; UT, untranslated.

FIG. 1. Comparison of various transfer vectors and their relation to p γ _{1360E}. The segment between the *Sal* I restriction sites of each vector named to the right is shown, with the size of the *Sal* I fragment indicated to the left. In every case, the remainder of the plasmid is identical to p γ _{1360E}. Note that each *Sal* I fragment is not drawn to scale. S, *Sal* I; RV, *Eco*RV; RI, *Eco*RI; P, *Pvu* I; H, *Hind*III; N, *Nco* I; K, *Kpn* I; Bam, *Bam*HI; Poly, polyhedrin; pA, polyadenylation site; kb, kilobase.

infected cells (4 μ g) by a CaCl_2 precipitation procedure. The resultant viral pool was screened, and occlusion-negative viruses were plaque purified as described (15).

Oligonucleotide-Directed Mutagenesis. All mutations were carried out on single-stranded DNA templates derived from phagemid constructs, essentially as described (18, 19). Oligonucleotides were synthesized on an Applied Biosystems model 380B DNA synthesizer. For deletions, nucleotides were designed as described (20).

Characterization of Proteins Produced by Virally Infected Cells. Total cellular lysates and supernatants were subjected to SDS/PAGE according to Laemmli (23). For studies involving the intrinsic [^{35}S]methionine labeling of cellular proteins, 1 μCi (1 Ci = 37 GBq) of [^{35}S]methionine was added to previously methionine-starved cells and incubated for 4 hr. Acrylamide gels were dried, and autoradiograms were produced, typically in 30-min exposures using Kodak XAR film and Kronex (DuPont) intensifying screens. Western blot analysis was carried out essentially as described (24), with an ^{125}I -labeled affinity-purified goat anti-mouse IgG antiserum.

Isolation of Intact, *p*-Azophenylarsenate (Ars)-Specific Immunoglobulin. IgG was purified from cell culture supernatants in a two-step purification. The pH of the supernatants was adjusted to pH 8 with 2 M Tris (pH 8.8). This mixture was then subjected to high-speed centrifugation ($10,000 \times g$) for 10 min and further clarified by filtration through a $0.45\text{-}\mu\text{m}$ filter (Millex-HV, Millipore). This solution was then passed over a protein G-Sepharose column (Pharmacia), washed in 20 mM phosphate (pH 7.0), and eluted with 0.1 M glycine (pH

2.7). The eluate was immediately neutralized with 2 M Tris (pH 8.8). This material was then concentrated to 1–10 mg/ml by centrifugation in a Centricon-30 (Amicon). A second purification step was carried out on an Ars-lysine-Sepharose column prepared as described (25). The protein G-reactive material was passed over a phosphate-buffered saline-equilibrated Ars column and washed in phosphate-buffered saline; Ars-specific immunoglobulin was eluted with 0.2 M arsanillic acid/0.2 M Tris (pH 8.2), then extensively dialyzed, and concentrated to 1 mg/ml.

Analysis of N-Linked Carbohydrate Addition. The addition of N-linked carbohydrate by infected insect cells was verified by culture in the presence of tunicamycin (5 $\mu\text{g/ml}$) to prevent the addition of N-linked carbohydrate (26, 27). Lysates were subsequently analyzed by SDS/PAGE as stated above.

Assays for Antibody Activities. Antibodies were tested for both the ability to bind arsonate and the expression of idiotypic determinants by solid-phase RIA methodologies as described (28). Results are expressed as the percent inhibition of binding, with the amount of protein used to achieve that inhibition.

RESULTS

Construction of Baculovirus Transfer Vectors. The baculovirus transfer vectors used in this study are depicted in Fig. 1. The starting point for all of the constructs is the plasmid pAc360 (15). pAc360 is a pUC8-based vector containing a 7.1-kilobase viral DNA insert, including a mutated *Autographa californica* polyhedrin gene and associated flanking DNA. To facilitate the construction and further manipulation of transfer vectors containing foreign genes, an origin of replication from the F1 filamentous phage was inserted into the *EcoRI* site of pAc360. An *Nco* I site was then introduced into the vector such that the initiation methionine codon of polyhedrin is coincident with the ATG in the *Nco* I recognition sequence (CCATGG). This intermediate vector is a generally useful transfer vector in that many eukaryotic genes possess an *Nco* I restriction site at their initiation codon. Furthermore, Kozak (29) has reported that the optimal eukaryotic ribosome recognition/initiation sequence contains this sequence. The immunoglobulin heavy chain from the *Ars*-specific hybridoma 93G7 (γ_1 , κ) was then cloned into this *Nco* I site, yielding pH γ_1 360E.

To express an intact immunoglobulin, both heavy and light chains need to be expressed in the same cell. Three general strategies were followed to try to achieve this double expression. The first was based on the double infection of insect cells by two recombinant viruses, each containing the heavy- or light-chain gene, respectively. To this end, a cDNA of the 91A3 hybridoma light chain was produced by using oligonucleotides to delete the introns from a rearranged κ light-chain gene cloned in our laboratory (17). This light-chain cDNA then had an *Nco* I site introduced at its initiation codon and was subcloned into pAc360E in a similar fashion as described above for the heavy chain, yielding pH κ -360.

The second strategy to coexpress two chains was based on the dicistronic expression of both polypeptides from one transcription unit. Kozak (29) has shown in an *in vitro* eukaryotic translation system that ribosomes can reinitiate translation of a second translation unit. On the basis of this observation, the 91A3 κ cDNA was inserted behind the 93G7 γ_1 cDNA. By means of site-specific mutagenesis, a unique *Xba* I restriction site was introduced 15 nucleotides 3' of the heavy-chain termination codon, and two *Xba* I restriction sites were introduced immediately flanking the 91A3 κ cDNA. Restriction of both constructs with *Xba* I and subsequent ligation led to a clone (pH $\gamma_1\kappa$ -360, Fig. 1) that contained the 93G7 heavy chain and the 91A3 light chain in a continuous transcription unit.

The third strategy was to create a recombinant virus with two polyhedrin promoters, each initiating transcription of either the heavy- or light-chain cDNA. This technique was shown to work previously in the expression of the lymphocytic choriomeningitis virus N protein as an independent transcription unit, 5' of the polyhedrin gene (30). In our case, a true double-recombinant transfer vector was engineered such that two foreign genes were contained in the same plasmid. The transfer vector pH $\gamma_1\kappa$ -Inv has two independent polyhedrin transcription units in opposite orientations, surrounded by the 5' and 3' flanking viral sequences necessary for the subsequent homologous recombination event to produce a recombinant virus. We did not attempt to create a transfer vector with both genes in the same orientation, since it is likely that one gene or the other would be deleted from the recombinant virus by means of homologous recombination.

Characterization of Recombinant Virus Protein Products. Recombinant viruses were analyzed for the identity and correct placement of the heavy- and/or light-chain genes within the viral genome by Southern filter-hybridization experiments (data not shown). Such analysis verified, in all cases, that the viruses produced contained the foreign gene(s) in both the correct location and orientation.

To characterize the protein products of virally infected cells, infected Sf9 cultures were intrinsically labeled with [³⁵S]methionine 48 hr postinfection, and total cell lysates were analyzed by SDS/PAGE. Lane 1 in Fig. 2 shows the pattern of proteins seen in uninfected cells, while lane 2 shows polyhedrin production by wild-type AcNPV-infected cells. Production of heavy chain by the recombinant virus pH γ_1 360E is seen as the $\approx$ 50-kDa band in lane 3 of Fig. 2, while production of light chain by pH κ -360 is seen as the $\approx$ 24-kDa band in lane 4 of Fig. 2 (large arrowheads). Both the heavy chain and light chain have leader peptides that are properly trimmed (see below), and for the light chain, both forms can be seen as a doublet in the cell lysates (small arrowhead). By using these same two viruses in a coinfection, both heavy and light chains are made (Fig. 2, lane 5; reduced), and intact immunoglobulin heterodimers are assembled (Fig. 2, lane 6; unreduced). Lanes 7 (reduced) and 8 (unreduced) of Fig. 2 demonstrate that heavy chains, light chains, and intact immunoglobulins are also produced by cells infected with the double-recombinant virus pH $\gamma_1\kappa$ -Inv. Note that the amount of intact immunoglobulin present in Fig. 2, lanes 6 and 8 is small compared to the amount of heavy and light chains seen in lanes 5 and 7 of Fig. 2, and in a reciprocal fashion, only small amounts of free heavy or light

FIG. 2. SDS/PAGE analysis of cellular lysates intrinsically labeled with [³⁵S]methionine. SDS lysates from $\approx 2 \times 10^5$ cells were electrophoresed through a 12% polyacrylamide Laemmli gel. The gel was treated with EN³HANCE (DuPont) and dried, and 6-hr autoradiographs were produced. The lanes contain total cellular lysates from uninfected Sf9 cells (lane 1) and from Sf9 cells infected with AcNPV (lane 2), pH γ_1 360E (lane 3), pH κ -360 (lane 4), both pH γ_1 360E and pH κ -360 (lanes 5 and 6), or pH $\gamma_1\kappa$ -Inv (lanes 7 and 8). Samples were reduced in lanes 1–5 and 7 and were unreduced in lanes 6 and 8. The positions of heavy chain (H), light chain (L), intact immunoglobulin (IgG), and polyhedrin polypeptides (P) are indicated by the arrowheads.

chains are seen in Fig. 2, lanes 6 or 8. The samples in each pair are identical with the exception of the addition of 2-mercaptoethanol in the reduced lanes. We believe that the paucity of intact antibody molecules in the cells is due to the active transport of whole immunoglobulins out of the cell, while individual or incorrectly associated chains (detected by Western blot, see below) are retained.

Western blot analysis of the proteins produced in infected cells is shown in Fig. 3. Note that only cells infected with immunoglobulin cDNA containing viruses produce a protein detected with an ¹²⁵I-labeled goat anti-mouse IgG antiserum. These data verify that the protein products seen in Fig. 2 are authentic immunoglobulin polypeptides. As noted above, the majority of intracellular immunoglobulin polypeptides appear to be associated in abnormal complexes. This is most strikingly evidenced in Fig. 3, lanes 7 and 8, where most of the immunoglobulin polypeptides, which behave as typical heavy and light chains under reducing conditions, are detected as abnormal complexes when unreduced.

The strategy of expressing both genes from a dicistronic construct was unsuccessful. Cells infected with pH $\gamma_1\kappa$ -360 express the heavy chain but no detectable light chain. The lack of κ expression was verified in several different constructs, wherein the distance between the heavy- and light-chain genes as well as the initiation context of the second gene were varied. One example is shown in Fig. 1 as pH $\gamma_1\kappa\Delta$ Gap. In all cases, Northern blot analyses indicated that a single message containing both genes was being transcribed at high levels in the infected cells. Thus only the first gene appears to be translated from a dicistronic message in Sf9 cells.

To assess whether the expected addition of an N-linked carbohydrate moiety is being accomplished, infected cells were incubated in the presence of tunicamycin, a specific inhibitor of the addition of N-linked oligosaccharides. Fig. 4 shows the shift in apparent molecular weight of the heavy-chain polypeptide (lanes 1 and 2) in tunicamycin-treated Sf9 cells. This clearly demonstrates that insect cells add an N-linked oligosaccharide to the heavy chain as expected. It is important to note that (where it has been analyzed) the subsequent carbohydrate modifications that are normally seen in higher eukaryotic cells are not seen in Sf9 cells. It is expected, then, that the oligosaccharides added to the heavy chain are not being trimmed and redecorated as would be done in a normal B cell.

Finally, to verify removal of the leader peptide of both the heavy and light chains, protein sequencing was employed on purified light and heavy chains. Fifty picomoles of each material was analyzed on an automated protein sequencer (Applied Biosystems model 477A). The sequences of both the heavy and light chains matched the expected sequences, and no phenylthiohydantoin amino acids representing leader peptide sequences could be identified.

Isolation and Functional Analyses of Recombinant Immunoglobulin Molecules. A two-stage purification of recombi-

FIG. 3. Western blot analysis of cellular lysates. Samples were electrophoresed as described for Fig. 2, electroblotted onto a poly(vinylidene difluoride) membrane (Millipore), and hybridized with an ¹²⁵I-labeled goat anti-mouse IgG antiserum. Lane assignments and marker positions are as for Fig. 2.

FIG. 4. Analysis of carbohydrate substitutions of recombinant molecules. SDS lysates from $\approx 2 \times 10^5$ cells were electrophoresed through a 9% polyacrylamide Laemmli gel. The gel was treated with EN³HANCE (DuPont) and dried, and 6-hr autoradiographs were produced. The lanes contain total cellular lysates from Sf9 cells infected with pH γ 13'Fix (lanes 1 and 2) or AcNPV (lanes 3 and 4). Cells were cultured without (lanes 1 and 4) or with (lanes 2 and 3) tunicamycin (5 μ g/ml). Arrowheads indicate the shift in molecular weight of a viral glycoprotein (gp64) or that of the recombinant heavy chain (H).

nant immunoglobulins from infected cell cultures was performed. The first step, a protein G affinity column, purified both the recombinant murine IgG and the fetal calf serum-derived bovine immunoglobulin. To achieve a final purification, the recombinant molecule's affinity for Ars was used in an affinity purification on Ars-lysine-Sepharose 4B. The material that eluted from this column is electrophoretically identical to ascites-derived 93G7 hybridoma protein. Fig. 5 shows the Coomassie blue staining pattern of material at each stage of purification. Western blot analysis (data not shown) of material like that shown in Fig. 5 verifies that the murine IgG copurifies with the Ars binding fraction, while the nonbinding fraction is devoid of murine antibody. By using such a purification scheme, IgG can routinely be recovered at a level of 5 μ g/ml of Sf9 monolayer culture supernatant.

To verify that the recombinant IgG functions as well as native murine IgG, competitive RIAs were performed to assay Ars binding, the expression of polyclonal cross-reactive idiotype (CRI_A), as well as several monoclonal idiotypes. The results from such an analysis (CRI_A expression) are depicted in Fig. 6. Clearly, the recombinant antibody (BV-wt) competes for binding to anti-idiotypic antibody (rabbit anti-CRI_A) identically to a CRI_A expressing hybridoma antibody (93G7). The irrelevant antibody NQ5 and the idiotype-negative anti-Ars antibody 91A3 serve as negative controls. Table 1 summarizes the results of several assays, wherein the recombinant antibody reacts exactly as would be predicted based on assays involving the parent antibody molecules (93G7 and 91A3). Thus the idiotypes associated

FIG. 5. SDS/PAGE analysis of recombinant antibody purification. Protein G affinity-purified material from a culture infected with pH γ 1 κ -Inv was loaded on a 12% reducing polyacrylamide gel (lane 1). Lane 2, fraction of this material that binds Ars; lane 3, non-Ars-binding fraction; lane 4, protein G-reactive material from an AcNPV-infected culture supernatant. Arrowheads indicate the position of the heavy (H) and light (L) chains.

FIG. 6. Unlabeled competitor RIA for the analysis of cross-reactive idiotype (CRI) expression by recombinant and hybridoma antibodies. Recombinant antibody BV-wt was compared to 93G7 (CRI⁺, anti-Ars), 91A3 (CRI⁻, anti-Ars) and NQ5 (CRI⁻, anti-phenyloxazalone) for their ability to compete with labeled 93G7 for binding to rabbit anti-CRI (F354)-coated plates. Error bars indicate the standard deviation obtained in three replicate assays.

primarily with the heavy chain, E4 and 2D3 [as determined in chain recombination experiments (21)], are similarly expressed by the recombinant antibody (BV-wt) and 93G7. In contrast, 7B7, a light-chain-associated idiotype, is not expressed by BV-wt as would be predicted based on the lack of expression of this idiotype by the 91A3 light chain (28).

DISCUSSION

The expression of a covalently associated immunoglobulin heterodimer as described above represents several advances in the baculovirus expression system as first described by Smith *et al.* (13). First, the development of a phagemid derivative of the baculovirus transfer vector affords the opportunity to easily make specific mutations to the vector with relation either to the inserted foreign gene or to the transcriptional regulatory elements of the polyhedrin gene itself. The introduction of an *Nco*I restriction endonuclease recognition sequence coincident with the initiation codon of the native polyhedrin gene is one example of the type of improvements that can be made to the expression vector. With a minimal number of changes to the 5' untranslated sequence of the polyhedrin gene, a broadly useful vector with high expression characteristics was developed. On the basis of previous work with the transfer vectors pAc373 and pAc610 (unpublished data), the minimal changes made in the 5' untranslated region of the polyhedrin gene in our vector resulted in a significant increase in the amount of protein expressed from otherwise identical inserted genes.

In contrast, changes at the 3' end of the polyhedrin transcription unit seem to have very little effect on the level of expression of inserted genes. For example, the heavy chain expressing vector pH γ 1360E has the polyadenylation signal and 3' untranslated (UT) region of the heavy chain included 5' of the polyhedrin gene (with its polyadenylation signal and 3' UT region). In this vector, we found that the mRNA produced contained both the entire heavy-chain

Table 1. Functional comparison of 93G7 and the recombinant antibody BV-wt

Antibody	Ars binding	CRI _A	Idiotype		
			2D3	7B7	E4
93G7	100 (1)	100 (0.1)	0 (>5)	100 (0.3)	100 (0.6)
BV-wt	100 (1)	100 (0.3)	0 (>5)	0 (>5)	100 (0.6)

The values given are the percent inhibition at the concentration of inhibitor given in parentheses (in μ g/ml).

sequence as well as that of the polyhedrin gene. This indicated that the insect cell transcriptional machinery clearly preferred the polyadenylation sequence of the viral gene over that of the heavy-chain gene. We found that deletion of the heavy-chain 3' UT region and the entire polyhedrin coding region (pH γ_1 3'Fix, Fig. 1) had no effect on the level of expression of the inserted gene. This experiment suggests that the presence of the polyhedrin gene and its immediate 3' UT region has little if any effect on the level of expression from the polyhedrin promoter. Given a choice, however, the viral polyadenylation signal is used over that from a heavy chain.

The expression of a heterodimer in a baculovirus expression system was previously described in a study of influenza virus polymerase (P) proteins (22). In that study, PB1 and PB2 were determined to be noncovalently associated in Sf9 cells coinfecting with separate recombinant viruses. The data reported here differ from this previous report in two important ways. First, immunoglobulins are covalent heterodimers, involving the assembly of a total four polypeptides into the final macromolecule. We have shown the assembly of such molecules to occur in insect cells, including correct interchain disulfide bonding, as evidenced by the purification of a molecule with all the correct biochemical and functional properties. The second important difference lies in our ability to express an immunoglobulin heterodimer from a single recombinant virus containing both genes. The idea for generating such a double-recombinant virus has been described (30), but our study represents the application of such a strategy to the expression of two foreign genes. While double-infection strategies suffice to express two genes in a single cell, double-recombinant viruses have several advantages. For instance, in situations where an equivalent stoichiometry of expression of the two proteins is desirable, a double-recombinant virus ensures an equal delivery of each transcription unit to every infected cell.

We also described the failure of an attempt to express two genes from a dicistronic message under control of the polyhedrin promoter. Although a complete dicistronic mRNA was shown to be produced, the second gene in the message was not translated at any detectable level despite several attempts to optimize the context between the stop codon of the first gene and the initiation codon of the second. Thus Kozak's report (29) of the ability of an *in vitro* eukaryotic translation system to initiate the second gene of a dicistronic message does not appear to extend to the *in vivo* situation in Sf9 cells.

Finally, the fact that these molecules behave identically to their ascites-derived counterparts suggests that the antibody molecules produced in an insect cell are identical to authentic mouse immunoglobulins. This finding is significant in terms of the production of large amounts of monospecific antibodies for experimental, diagnostic, or even therapeutic purposes.

The production of recombinant antibodies *in vitro* has a broad variety of applications in both basic structure/function studies as well as in diagnostic and therapeutic applications. Although the needs of functional studies have been met in many laboratories by transfection experiments, the yields of antibodies from these systems are modest and insufficient for structural analysis or therapeutic use. Bacterial expression of immunoglobulin fragments has been used to achieve a higher yield for structural studies, but these truncated molecules do not possess the biological functions associated with the constant domains of the immunoglobulin molecule. Even if entire immunoglobulin molecules were to be expressed in a bacterial system, the lack of carbohydrate addition would no doubt preclude their use in most therapeutic situations. We

have described the high-level expression of functional, glycosylated immunoglobulin heterodimers. These antibody molecules are secreted into the supernatant and are easily purified.

We thank Max Summers for supplying us with the reagents and information needed to begin our baculovirus studies. We also thank Jacques Urbain and Al Nisonoff for providing Ab2 antibodies; John Sims and Terry Rabbitts for the original cloning of the 93G7 heavy chain; and Inaki Sanz for the cloning of the 91A3 light chain. We also thank Kathryn Meek for her numerous insightful additions to the experimental process. J.D.C. was supported in part by grants from the National Institutes of Health (AI-12127 and CA-44016) and the Robert Welch Foundation.

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