

# Expression of a human, neutralizing monoclonal antibody specific to Puumala virus G2-protein in stably-transformed insect cells

Mary C. Guttieri\*, Carol Bookwalter, Connie Schmaljohn

Virology Division, United States Army Medical Research Institute of Infectious Diseases, Bldg. 1301, Fort Detrick, Frederick, MD 21702-5011, USA

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## Abstract

We cloned the heavy- and light-chain antibody genes of a human X (human×mouse) trioma secreting a neutralizing, IgG monoclonal antibody to the G2-protein of Puumala virus. The antibody genes were inserted separately into plasmid transfer vector pIEI-4 such that the genes were under control of the baculovirus immediate early gene promoter, IEL. *Trichoplusia ni* (TN) cells were co-transfected with these constructs and a selection plasmid containing a neomycin-resistance gene. Cloned transformants expressing the IgG monoclonal antibody were identified by ELISA of transfected TN cell culture supernatants. TN cell lines were established from four selected clones, of which one was chosen for detailed analysis. Specificity of the insect cell-expressed human antibody was determined by ELISA with Puumala virus-infected cell lysates and by immune-precipitation of radiolabeled Puumala virus proteins. The expressed IgG retained the ability to neutralize Puumala virus in plaque-reduction neutralization assays. Using competitive polymerase chain reaction methods, multiple copies of integrated heavy- and light-chain antibody genes were detected in the insect cell genome. The transformed insect cells were stable and continuously expressed biologically active IgG. We conclude that this methodology provides an alternative eukaryotic source for the generation of human antibodies. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Puumala virus; Monoclonal antibody; Insect cell; Stable transformation

**Abbreviations:**  $\beta$ -gal,  $\beta$ -galactosidase; ELISA, enzyme-linked immunosorbent assay; EMEM, Eagle's minimal essential medium; FBS, fetal bovine serum; HFRS, hemorrhagic fever with renal syndrome; HTNV, Hantaan virus; MAbs, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PBS-T, PBS with Tween-20; PBS-TS, PBS-T with skim milk; PCR, polymerase chain reaction; PRNT, plaque-reduction neutralization test; PUUV, Puumala virus; TN, *Trichoplusia ni*.  
\*Corresponding author. Tel.: +1-301-619-4100; fax: +1-301-619-2439.

E-mail address: mary.guttieri@det.amedd.army.mil (M.C. Guttieri).

## 1. Introduction

Hemorrhagic fever with renal syndrome (HFRS) is a severe and sometimes fatal disease caused by certain viruses in the *Hantavirus* genus of the *Bunyaviridae* family. Rodents are the natural hosts of hantaviruses, and transmission of virus to humans occurs via aerosolized urine, feces, and saliva of infected animals (Lee et al., 1982; Lee and Johnson, 1982). HFRS is caused by at least four distinct

hantaviruses: Hantaan virus (HTNV), Puumala virus (PUUV), Seoul virus, and Dobrava-Belgrade virus (Lee and Dalrymple, 1989; Antoniadis et al., 1996). To date, the primary treatment for HFRS is critical care management, often including hemodialysis (Van Ypersele de Strihou and Mery, 1989). Although the drug ribavirin statistically improves survival rate, it must be administered at an early stage of infection to successfully alter disease outcome and, as a result, is often ineffective for HFRS patients presenting with acute illness (Huggins et al., 1991). HFRS poses a significant health threat, with severe and sometimes fatal forms of disease reported in epidemic and endemic proportions throughout Asia and Europe (Kanerva et al., 1998). Due to its widespread dissemination, HFRS has historically represented a significant problem for military personnel (Kanerva et al., 1998; Markotic et al., 1996), further strengthening the need for therapeutic measures for the treatment or prevention of this disease.

There have been no published reports of controlled clinical trials of immune-therapy for HFRS. However, studies in hamsters and rats indicated that passive transfer of neutralizing monoclonal antibodies (MAb) or polyclonal sera can passively protect animals from challenge with hantaviruses (Zhang et al., 1989; Schmaljohn et al., 1990; Arikawa et al., 1992). Additional studies demonstrated that a G2-specific neutralizing MAb administered up to 4 days after challenge with HTNV can cure hamsters of infection (Liang et al., 1996). Furthermore, analysis of blood collected from HFRS patients indicated that patients remain viremic during the acute phase of disease (Antoniadis et al., 1987; Juto et al., 1997; Rollin et al., 1995; Yao et al., 1989). As a result, these collective data provide rational reasons to suspect that administering human neutralizing antibodies to HFRS patients may effectively limit viremia and, thereby, disrupt the natural course of disease. Presently, there is no evidence to indicate that immune-intervention for HFRS is detrimental to disease management, especially concerning potential complications associated with immune-complex formation. Although immune complexes have been detected in circulation of HFRS patients, various studies of PUUV-induced HFRS patients detected no correlation between immune-complex formation and disease pathogenesis (Jokinen et al., 1977, 1978;

Penttinen et al., 1981; Temonen et al., 1996). However, recent studies of complement activation in PUUV-infected patients indicated pronounced activation via the classical pathway in acutely ill patients, suggesting a correlation between complement activation by immune complexes or by viral components and the clinical severity of disease (Paakkala et al., 2000). Therefore, consideration should be given to the possible risk of immune-related pathogenesis when devising immune-therapeutic strategies for the treatment of PUUV-induced HFRS.

To explore the potential of immune-therapy for HFRS, we investigated various methodologies by which hantavirus-specific human antibodies can be generated. In a previous study, we used a baculovirus expression system to generate a recombinant baculovirus expressing a neutralizing human IgG MAb to PUUV (Liang et al., 1997). Although high levels of the IgG were produced from this recombinant, the infection culminated in death of the host cell, thereby allowing for only transient expression of the antibody. Previous studies also suggested that, during the late phase of baculovirus infection, the host's secretory pathway can become impaired (Jarvis and Summers, 1989). To overcome the potential problems associated with baculovirus expression, we report here an alternate method that allows for the continuous expression of antibody genes from transformed insect cell genomes. The genes are inserted into the insect cell chromosome under control of the baculovirus immediate early gene promoter, IE1, which is recognized by the insect cell RNA polymerase (Jarvis et al., 1990, 1996).

## 2. Materials and methods

### 2.1. Virus and cells

PUUV, strain K27 (Tkachenko et al., 1984), was propagated in Vero-E6 cells (Vero C1008; ATCC CRL 1586) with Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics as previously described (Schmaljohn et al., 1983). *Trichoplusia ni* (TN) cell line BTI-TN-5B1-4 (Invitrogen, Carlsbad, CA) was used to establish transformed insect cell lines. TN cells were maintained as stationary cultures at 28°C

in serum-free Excell-405 medium (JRH Biosciences, Lenexa, KS) supplemented with antibiotics (Summers and Smith, 1987). For insect cell transformations, cells were propagated in TNMFH medium (Summers and Smith, 1987) supplemented with 10% FBS and antibiotics (complete TNMFH). The human X (human×mouse) trioma (hybridoma cell line IC9) secreting neutralizing G2-specific MAb to PUUV was described previously (Gustaffson et al., 1991; Lundkvist et al., 1993). Hybridoma cells were cultured in monolayers in OptiMEM medium (Gibco BRL Life Technologies Inc., Gaithersburg, MD) supplemented with 6% FBS.

## 2.2. Construction of transfer vectors for integration of heavy and light chain antibody genes

The heavy- and light-chain antibody genes of human hybridoma IC9 were cloned previously (Liang et al., 1997). Briefly, cDNA was synthesized with total cellular RNA, oligo dT primers, and reverse transcriptase. Polymerase chain reaction (PCR) and specific heavy- and light-chain primers were used to amplify cDNA, which was then cloned into pCR II (Invitrogen). Transfer vector pIEI-4 (Novagen Inc., Madison, WI), which contains a multiple cloning site downstream of the baculovirus immediate early gene promoter, IEI, (Jarvis et al., 1996), and selection plasmid pIEI-neo (Novagen), which confers neomycin resistance, were used to generate transformed insect cell lines. To construct transfer vectors for expression of the PUUV-specific antibody genes, pCR II *Bam*HI and *Bgl*II fragments containing the complete heavy- and light-chain genes, respectively, were subcloned separately into the *Bam*HI site of pIEI-4.

## 2.3. Transformation of insect cells

Using Lipofectin reagent (Gibco BRL) and previously established methods (Jarvis et al., 1990; Joyce et al., 1993), we transformed TN cells by cotransfection with pIEI-neo and the pIEI-4 transfer vectors containing the PUUV-specific heavy- and light-chain antibody genes. To allow for selection of neomycin-resistant cells, transfected cells were propagated for 2 weeks in complete TNMFH supplemented with the

antibiotic G418 (Gibco BRL). After this initial selection, cell cultures were maintained in medium lacking the neomycin antibiotic. To establish clonal cell lines, individual colonies were picked and amplified in complete TNMFH until sufficient cell concentrations were present for analysis. Clonal cell lines were maintained as stationary cultures at 28°C in Excell-405 medium.

## 2.4. Purification of IgG

To purify secreted IgG, supernatants from transformed insect cell cultures, grown as monolayers in 75-cm<sup>2</sup> flasks, were harvested 72 h after cell passage. The supernatant from cultured hybridoma cell line IC9 was collected after cells reached confluency (approximately 1 week). The insect and hybridoma cell culture supernatants were clarified by centrifugation and filtration with 0.45 µm filters then concentrated by using Centricon-10 filters (Amicon, Beverly, MA). IgG was recovered from concentrated supernatants by using an antibody affinity purification kit, MAb Trap GII (Amersham Pharmacia Biotech, Piscataway, NJ), and eluted fractions were concentrated with Centricon-10 filters. Protein concentration was determined by comparison to Coomassie blue-stained bovine serum albumin protein standards and by using the BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer's directions. Approximately 5 µg of each IgG preparation was examined by SDS–polyacrylamide gel electrophoresis (PAGE) in 12.5% acrylamide/DATD gels as previously described (Schmaljohn et al., 1983).

## 2.5. Enzyme-linked immunosorbent assay

Clonal cell lines secreting complete IgG were detected by capture enzyme-linked immunosorbent assay (ELISA) of insect cell culture supernatants. To do so, goat anti-human Fab-specific antibody (Sigma, St. Louis, MO) was diluted 1:1600 in phosphate-buffered saline (PBS), pH 7.4; and 100 µl was added to each well of flat-bottomed 96-well microtiter plates then incubated overnight at 4°C. The plates were washed one time with PBS containing 0.05% Tween-20 (PBS-T) and blocked for 1 h at room temperature with PBS-T containing 3%

skim milk (PBS-TS). After washing three times with PBS-T, 100  $\mu$ l of two-fold dilutions of insect cell culture supernatants in PBS-TS were added to the wells. The plates were incubated for 1 h at 37°C then washed four times with PBS-T. A 1:1600 dilution of horseradish peroxidase-conjugated, anti-human Fc-specific antibody (Sigma) was added and incubation was continued for 1 h at 37°C. After washing as above, TMB peroxidase substrate (Kirkegaard and Perry, Gaithersburg, MD) was added, plates were incubated at room temperature for 15 min, and results were measured at 450 nm using an automated microplate reader (Bio-Rad, Hercules, CA).

Antigen-binding characteristics of insect cell- and hybridoma-secreted antibodies were compared by indirect ELISA, using PUUV-infected cell lysates that were prepared as previously described (Chu et al., 1994). Briefly, flat-bottom 96-well microtiter plates were coated overnight at 4°C with antigen diluted 1:500 in PBS, pH 7.4. After blocking for 1 h at room temperature with PBS-TS, 100  $\mu$ l of two-fold dilutions of 1.28  $\mu$ g/ml preparations of the purified insect cell- or hybridoma-expressed IgG in PBS-TS were added to the wells. The ELISA was then continued as described above.

#### 2.6. Immune-precipitation of Puumala virus proteins

To compare antigen specificity, purified insect cell- and hybridoma-expressed antibodies were used to immune-precipitate radiolabeled authentic PUUV proteins from infected cell lysates. Radiolabeling, immune-precipitation, and gel electrophoresis were performed as previously described for HTN virus (Arikawa et al., 1989).

#### 2.7. Plaque-reduction neutralization test (PRNT)

Neutralization assays were performed as described previously (Liang et al., 1997), with the following modifications. Purified antibodies from hybridoma cells and transformed insect cells were standardized to 7.5  $\mu$ g/ml and then serially diluted in four-fold increments. Diluted IgG was incubated with 100 PFU of PUUV at 4°C overnight. Virus-antibody mixtures were applied to Vero-E6 cell monolayers in six-well cell culture plates and incubated at 37°C for

1 h. Monolayers were then overlaid with EMEM containing 0.6% agarose (Seakem ME, FMC Corp.; Rockland, ME). After incubation for 4 days at 37°C, a second overlay identical to the first was added to the plates, and incubation was continued until day 7 at which time a third overlay, containing neutral red (final concentration 0.167 mg/ml), was added. After incubation at 37°C for 24–48 h, plaques were counted, and the % reduction in plaque formation was determined by comparison with control wells containing cells infected with PUUV incubated with bovine serum albumin.

#### 2.8. Analysis of IgG expression from transformed insect cells

To measure IgG accumulation in transformed insect cell culture supernatants, 25-cm<sup>2</sup> flasks were each seeded with approximately  $2 \times 10^6$  TN cells and incubated without medium change at 28°C for 1–4 days. Supernatant was collected for analysis from one flask every 24 h. Cell viabilities were calculated for each collection point by trypan blue staining, and the concentration of IgG present in supernatants was determined by capture ELISA, using purified insect cell-expressed IgG as a standard. Briefly, twofold dilutions of insect cell culture supernatants or purified IgG were applied to wells of microtiter plates coated overnight at 4°C with a 1:1600 dilution of anti-human Fab antibody (Sigma). The ELISA was then carried out as described above. The quantity of secreted IgG for each time point was estimated by using the following, previously established, formula: ng IgG/cell = (the IgG dilution required to yield OD<sub>450</sub> of 0.4–0.5) (ng/ml of purified IgG required to yield OD<sub>450</sub> of 0.4–0.5) (ml of supernatant for each time point)/number of cells (Liang et al., 1997).

To determine the stability of IgG expression from transformed insect cells, supernatants were collected from cultured cells after 1, 5, 10 and 15 passages and then analyzed for the presence of IgG by ELISA. Cells were seeded at a concentration of  $1 \times 10^6$  TN cells per 25-cm<sup>2</sup> flask at each passage. Approximately 72 h after passage, supernatants were collected, cell viabilities were determined, and the concentration of IgG in each was measured as described above.

## 2.9. Competitive PCR

To estimate the copy number of integrated heavy- and light-chain antibody genes, genomic DNA from transformed TN cells was analyzed by competitive polymerase chain reaction (PCR) methods similar to those previously described (Gilliland et al., 1990). DNA manipulations, including extraction of insect genomic DNA, were carried out by standard techniques (Sambrook et al., 1989). For light-chain analysis, the competitor plasmid, pCL, was constructed by excising a 39 bp *ApaI* fragment within the light-chain gene contained in pCR II. The competitor plasmid, pCH, for heavy-chain analysis was constructed by removing an 89 bp *ApaI/AgeI* fragment within the heavy-chain gene contained in pCR II. PCR primers were designed to flank these excised regions to allow for coamplification of genomic and competitor DNAs, with competitive plasmid DNA distinguished from genomic DNA by size comparison of PCR products. The forward and reverse light-chain PCR primers used were 5'-TCTCCTCTCCTCCTCACTCTCCTCGCTCAC - 3' and 5'-TCAGCTTGGTCCCTCCGCCGAACACC-CAAG-3', respectively. For heavy-chain gene amplification, the forward and reverse primers were 5' - GCAAATGAACAGCCTCAGAGCCGAGGACAC-3' and 5'-GATTACGTTGCAGATGTAGGTCTGGGTGCCCA-3', respectively. Each PCR reaction mixture contained 1×PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl); dNTPs (200 μM each dATP, dCTP, dGTP, dTTP); 2.5 mM MgCl<sub>2</sub>; 20 pm each forward and reverse primer; 2.5 units of *Taq* polymerase, AmpliTaq (Perkin Elmer, Foster City, CA); and H<sub>2</sub>O to a final volume of 100 μl. Transformed genomic insect cell DNA was added to a master mix comprised of these components, allowing for a final concentration of genomic DNA per reaction mixture of 1 or 2 ng. A total of 90 μl of this mixture was then distributed into each of ten tubes containing 10 μl of competitor plasmid DNA from a dilution series, with final competitor DNA concentrations of 1, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015, 0.0075, 0.004 and 0.002 pg. Control reactions included samples in which either plasmid or genomic DNA were present and reactions in which DNA was completely omitted. Amplification was performed by 40 repeated PCR cycles at 94°C for 1 min, 62°C for

1 min, and 72°C for 2 min using the DNA thermal cycler 480 system (Perkin-Elmer). An aliquot of each reaction mixture was subjected to electrophoresis in 1% agarose/2% NuSieve gels, and the resultant PCR products were analyzed and compared by densitometry using the IS-1000 Digital Imaging System (Alpha Innotech Corp., San Leandro, CA). The copy number of input competitor plasmid DNA was determined by using a computer program that estimates the copy number of cloned DNA in a plasmid vector according to the following formula: copy number = Avogadro's number ÷ g/mol of cloned DNA. For assay of genomic copy number, the data were plotted as the log ratio of competitor DNA/genomic DNA vs. the log of input competitor DNA.

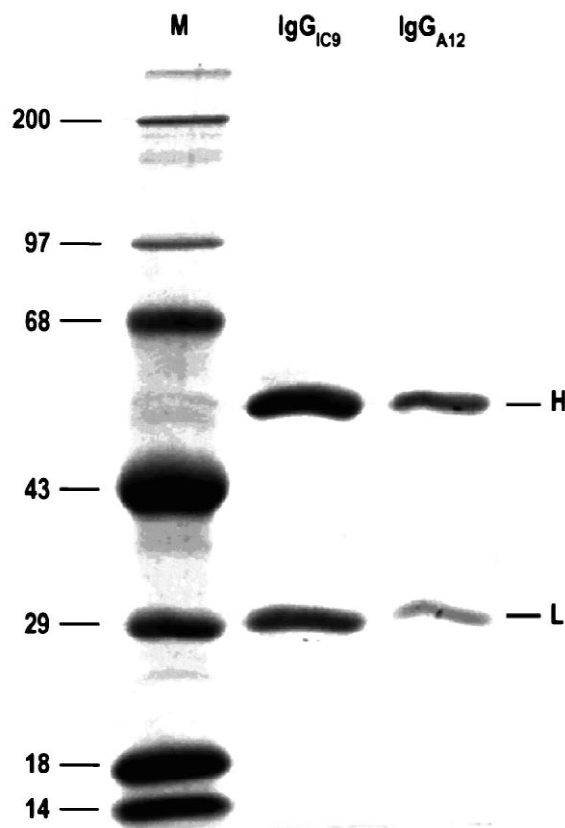


Fig. 1. Polyacrylamide gel electrophoresis and Coomassie blue staining of purified IgG. Antibodies were concentrated and affinity-purified from the supernatants of cultured IC9 hybridoma cells (IgG<sub>IC9</sub>) or A12 insect cells (IgG<sub>A12</sub>). The heavy (H) and light (L) chains are indicated. The sizes of molecular weight markers (M) are shown to the left of the panel.

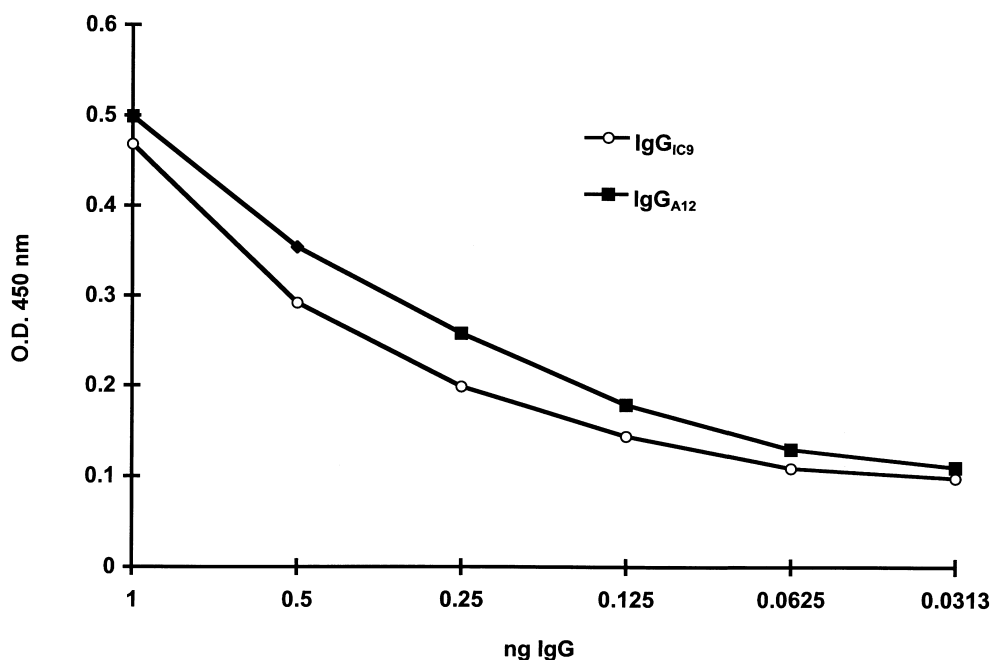


Fig. 2. Comparison by ELISA of the antigen-binding characteristics of IC9- and A12-expressed antibodies to PUUV. Indirect ELISA was performed with PUUV-infected cell lysate and twofold dilutions of affinity-purified IgG that was collected from the supernatant of cultured IC9 hybridoma cells (IgG<sub>IC9</sub>) or A12 insect cells (IgG<sub>A12</sub>).

A low DNA mass ladder (Gibco BRL) was used as a mass standard in ethidium bromide-stained gels.

### 3. Results

#### 3.1. Production and initial characterization of stably-transformed insect cell lines expressing IgG

Previously, we cloned the complete heavy- and light-chain antibody genes of a hybridoma, designated IC9, that secretes a human, neutralizing MAb to PUUV (Liang et al., 1997). Using the transfer vector pIEI-4 (Novagen), we constructed two plasmids that contained either the light- or heavy-chain gene under the control of the baculovirus promoter, IEI. To generate stable transformants, we cotransfected *Trichoplusia ni* insect cells with both plasmids and the selection plasmid pIEI-neo (Novagen) then propagated the cells initially under selective pressure with the antibiotic G418. TN cell clones that express IgG were identified by ELISA of insect cell culture supernatants. Of the numerous antibody-secreting

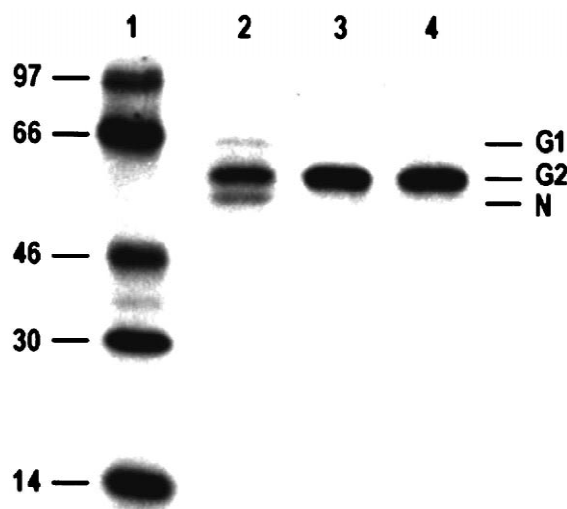


Fig. 3. Immune-precipitation and polyacrylamide gel electrophoresis of radiolabeled PUUV proteins. PUUV-infected Vero E6 cells were radiolabeled with <sup>35</sup>S-cysteine, and viral proteins were immune-precipitated with (2) polyclonal rabbit sera to PUUV; (3) purified IgG from the supernatant of cultured IC9 hybridoma cells; (4) purified IgG from the supernatant of cultured A12 insect cells. Nucleocapsid (N) and the glycoproteins, G1 and G2, are indicated to the right of the panel, and the sizes of molecular weight markers (1) are indicated on the left.

cells detected, four colonies were chosen for expansion based upon their high ELISA titers and stable growth in culture (data not shown). Initial examination of the four lines indicated that they had similar antibody secretion characteristics; therefore, only one cell line, A12, was further analyzed.

To confirm antibody secretion and to examine the integrity of the expressed protein, IgG was affinity-purified from A12 cell culture supernatants, then examined by SDS–PAGE. Heavy- and light-chains of the expected sizes were observed when compared to those of the hybridoma-expressed IgG (Fig. 1).

### 3.2. Functional analysis of the insect cell-expressed IgG

We compared the antigen-binding properties of the insect cell-expressed MAb, A12, to the hybridoma-expressed MAb, IC9, by ELISA. Both antibodies reacted with PUUV antigen similarly, with approximately 1 ng of either antibody required to yield an absorbance of 0.4–0.5 at OD<sub>450</sub> (Fig. 2). We confirmed the antigen specificity of the insect cell-

expressed IgG by immune-precipitation of radio-labeled PUUV proteins. Both the A12 and IC9-secreted MAbs precipitated G2 (Fig. 3). The neutralizing properties of the insect cell-expressed IgG were examined by plaque-reduction neutralization tests. The A12-expressed MAb retained the ability of the hybridoma-expressed IgG to neutralize PUUV in cell culture, with an 80% reduction in plaques resulting from the addition of approximately 0.3 µg of either MAb (Fig. 4).

### 3.3. IgG expression from transformed insect cells

To analyze IgG expression from A12 insect cells, we measured IgG accumulation in cell culture supernatants collected every 24 h for 1–4 days. Antibody concentration was determined by capture ELISA with purified A12-derived IgG as a standard. The quantity of IgG in A12 supernatants increased up to 72 h after cell passage, with a maximum accumulation of 58 ng of IgG per ml of cell culture supernatant (Fig. 5). We examined the stability of antibody expression by ELISA of A12 supernatants collected

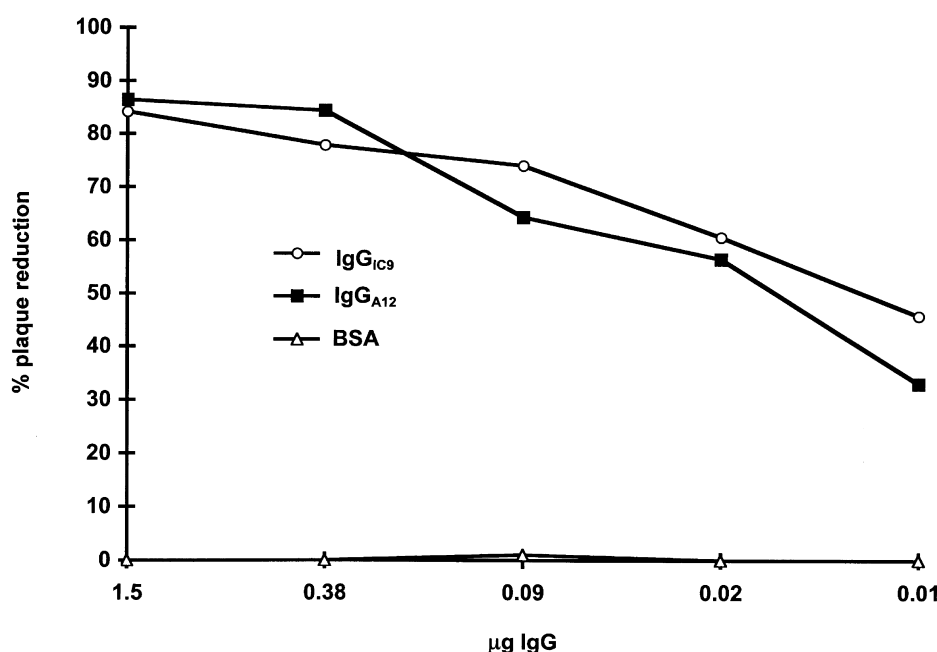


Fig. 4. Comparison of the neutralizing properties of IC9- and A12-expressed antibodies by plaque-reduction neutralization tests. Four-fold dilutions of affinity-purified IgG collected from the supernatant of cultured IC9 hybridoma cells (IgG<sub>IC9</sub>) or cultured A12 insect cells (IgG<sub>A12</sub>) were assayed by PRNT. Bovine serum albumin (BSA) was included in the study as a negative control.

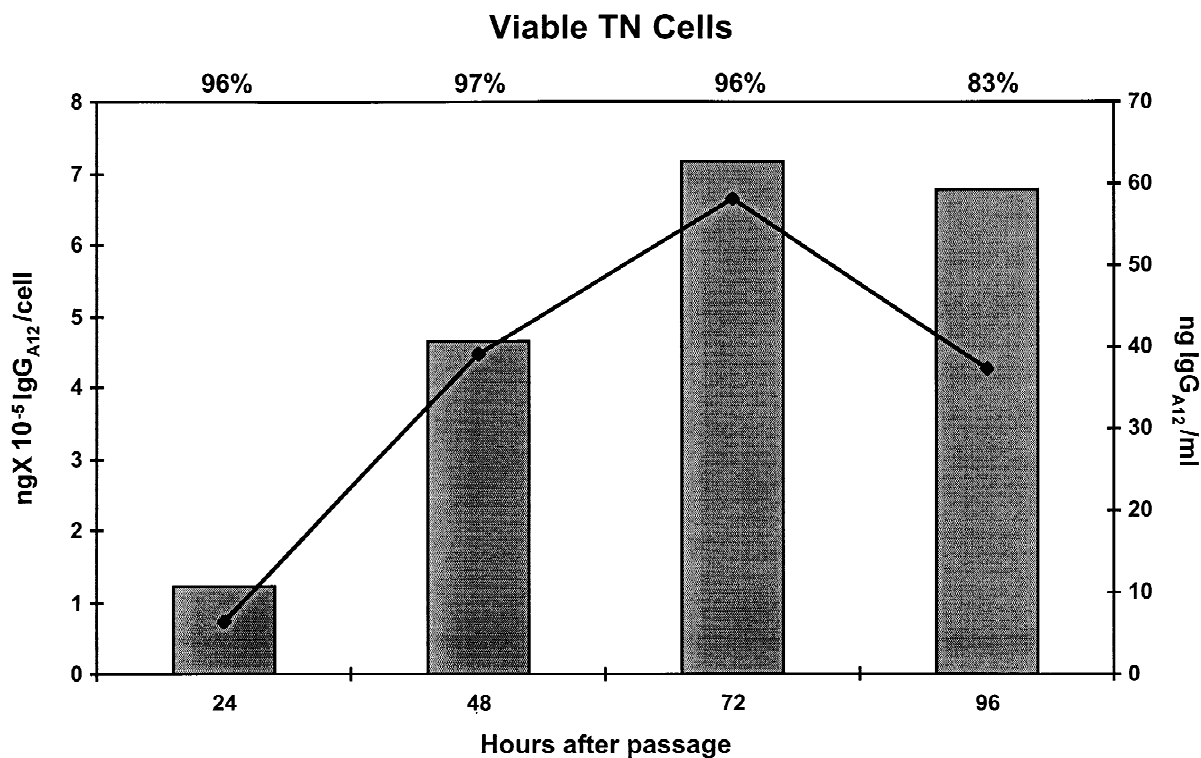


Fig. 5. Time course analysis of IgG accumulation in transformed insect cell culture supernatants. A12 insect cell culture supernatants were collected from 25-cm<sup>2</sup> flasks every 24 h for 4 days and were analyzed by capture ELISA to determine the concentration of accumulated IgG. Cell viabilities at each time point are shown above the graph, with collection points (hours after passage) displayed below the graph. The estimated amount of IgG per A12 insect cell ( $\text{ng} \times 10^{-5} \text{ IgG}_{\text{A12}}/\text{cell}$ ) is indicated by the bar graph, and the amount of IgG per ml of supernatant ( $\text{ng IgG}_{\text{A12}}/\text{ml}$ ) is indicated by the line graph.

from cultured cells after 1, 5, 10 and 15 passages. After an initial increase in antibody concentration, a stable IgG concentration of approximately 60 ng per  $10^6$  insect cells was detected (Fig. 6).

#### 3.4. Copy number of integrated light and heavy chain antibody genes

Jarvis et al. (1990) demonstrated by Southern hybridization analysis that foreign genes are inserted at multiple sites within the insect cell genome. To assay the copy number of heavy- and light-chain antibody genes within the genome of A12 insect cells, we used a competitive PCR method (Gilliland et al., 1990). By using PCR and densitometry, genomic DNA from A12 insect cells was titrated against a dilution series of competitive plasmid DNA

containing known copy numbers of the antibody genes. For our analysis, we constructed two competitive plasmids, pCL and pCH, that contained altered copies of the light- and heavy-chain genes. To discriminate competitive from genomic PCR products in ethidium-stained gels, 39 bp was deleted from the light-chain gene and 89 bp was deleted from the heavy-chain gene within the competitive plasmids. Co-amplification of pCL and A12 genomic DNA yielded 328 bp and 367 bp products, respectively (Fig. 7A). Analysis of the data revealed a genomic light-chain copy number of 258 (Fig. 7B). Amplification products of 389 bp and 300 bp resulted from competitive PCR with A12 and pCH DNA, respectively (Fig. 7C), and subsequent analysis indicated 287 copies of the integrated heavy-chain gene (Fig. 7D).



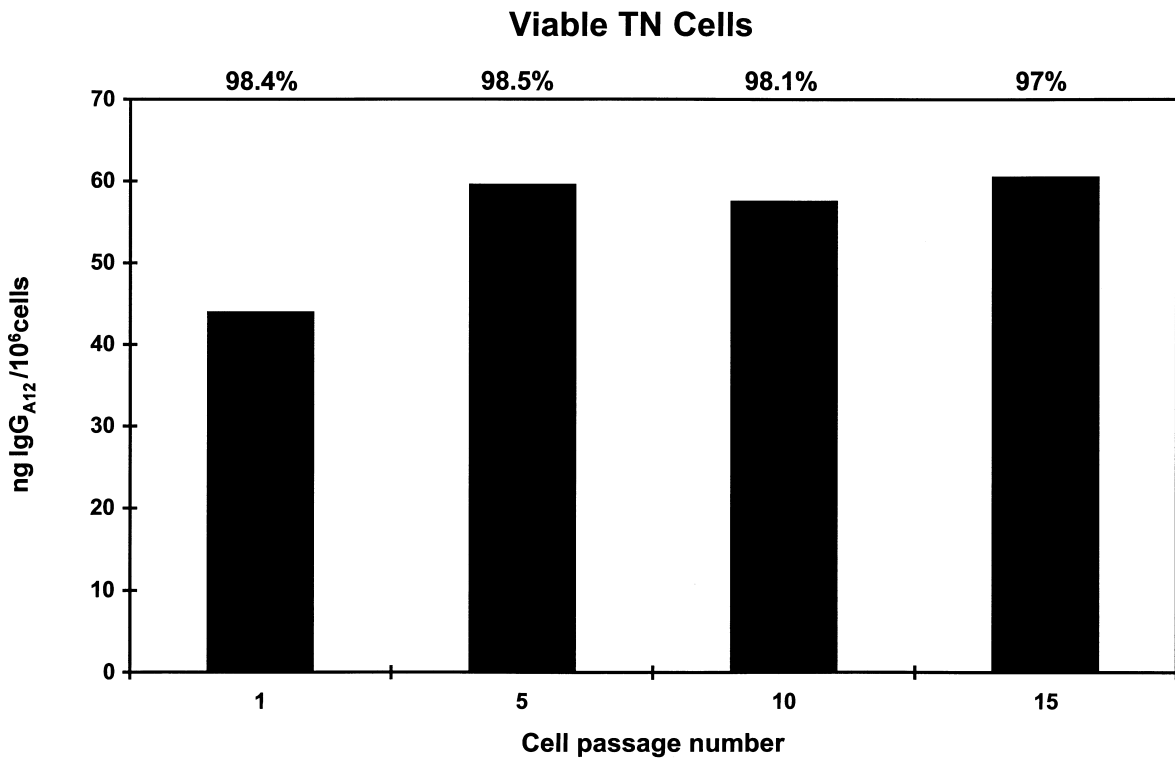


Fig. 6. Assay of stability of IgG expression from transformed insect cells. IgG in A12 cell culture supernatants collected after 1, 5, 10 and 15 passages was analyzed by capture ELISA. Cell viabilities and collection points (cell passage number) are shown above and below the graph, respectively.

#### 4. Discussion

In these studies, we generated a transformed insect cell line that expressed the same PUUV-specific IgG generated by infection with a baculovirus recombinant reported on previously (Liang et al., 1997). The insect cell-expressed antibody was biologically active, retaining the ability to bind PUUV G2 protein and to neutralize PUUV in cell culture. Furthermore, expression was continuous and stable.

Our analysis revealed multiple copies of heavy- and light-chain antibody genes within the insect cell genome. The copy numbers are comparable to those reported for transformed mammalian cell lines in which as many as 600 copies of the gene of interest were observed (Werner et al., 1998). Despite the presence of numerous copies of antibody genes, the concentration of IgG recovered from the transformed, insect cell culture medium was considerably

lower (approximately 0.06  $\mu\text{g}$  of IgG/ml) than the level generated by infection with our baculovirus recombinant (approximately 9  $\mu\text{g}$  of IgG/ml) (Liang et al., 1997) and with the predicted yield from hybridoma cells (1–10  $\mu\text{g}$  of IgG/ml) (Yokoyama, 1991). This observation is similar to results of a previous study that analyzed foreign protein production in transformed insect cells containing a  $\beta$ -galactosidase gene ( $\beta$ -gal) (Jarvis et al., 1990). Only 0.04–2  $\mu\text{g}/\text{ml}$  of  $\beta$ -gal was produced in transformed cells whereas approximately 200  $\mu\text{g}/\text{ml}$  was obtained in cells infected with a baculovirus recombinant expressing the same gene (Jarvis et al., 1990). This discrepancy may reflect differences between transcriptional activity from the IE1 promoter used for expression in transformed cells and the very strong polyhedrin promoter required for baculovirus expression. Furthermore, unidentified factors associated with integration may also have contributed to a

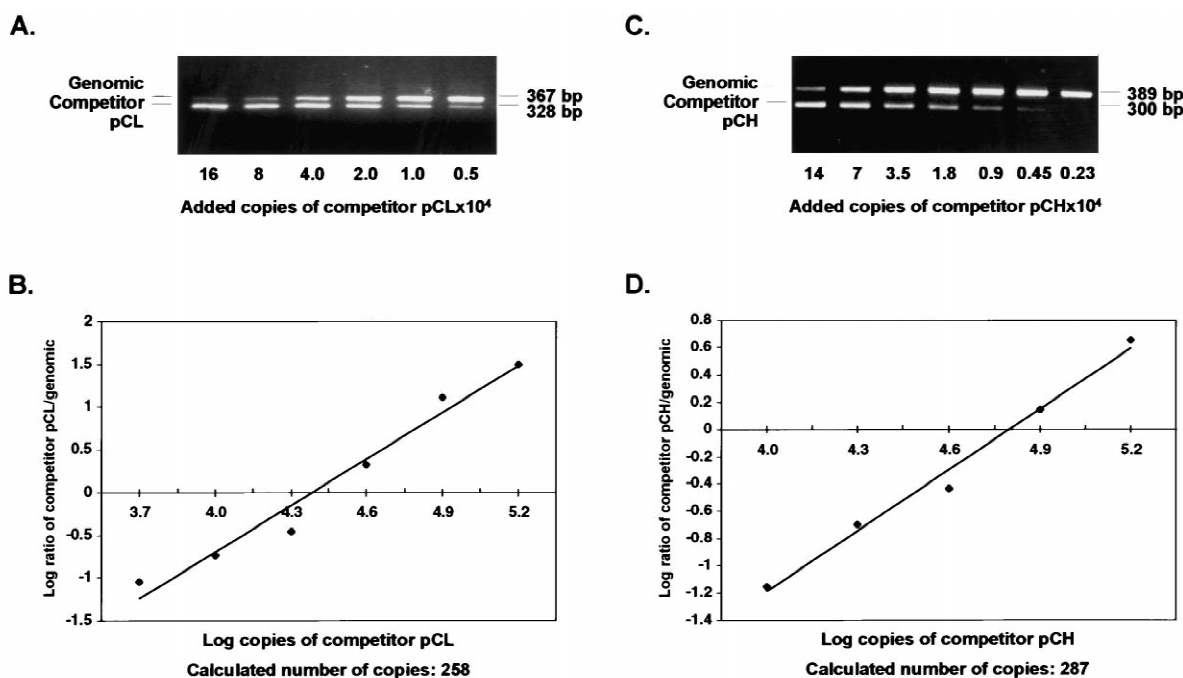


Fig. 7. Assay of genomic copy number of integrated light- and heavy-chain antibody genes by competitive PCR. (A and C) Agarose gel electrophoresis of PCR products resulting from co-amplification of TN insect genomic DNA and competitor plasmid pCL or pCH. Sizes of amplified products are shown to the right of the each figure, and the copy numbers of input competitor DNA for each PCR reaction mixture are indicated below the figures. (B and D) Densitometry data are plotted as the log ratio of competitor pCL or pCH DNA/TN genomic DNA vs. log copies of input competitor pCL or pCH DNA.

reduction in the level of expression. The mechanism of integration of genes into the insect cell genome and the location of insertion sites were not characterized. In addition, there have been no investigations to identify possible rearrangements or deletions of foreign coding sequences subsequent to integration. Therefore, the regulation of expression of inserted genes and, more specifically, factors that may down-regulate transcription from the IEI promoter are not known. As more is learned of the molecular biology of both the insect cell genome and baculoviruses, it may be possible to enhance transcription, thereby improving the yield of foreign proteins obtained from transformed cell cultures. It may become possible to provide *cis*- or *trans*-acting sequences that will serve to up-regulate transcription after integration has occurred and/or that will improve integration, guiding insertion to sites favorable for transcription.

Insect cell expression systems offer an attractive

alternative to bacterial and yeast systems as foreign proteins generated in insect cells are correctly folded and processed (Verma et al., 1998). When compared to mammalian expression systems, baculovirus- and insect cell-mediated expression systems provide a safer means to produce therapeutic proteins. Baculoviruses have a very limited host range, and insect cells do not possess factors, associated with mammalian cells, that can be potentially harmful to humans (e.g., retroviral elements etc.). Because insect cells are unable to accomplish all of the carbohydrate modifications exhibited by mammalian cells, baculovirus- or transformed insect cell-expressed antibodies may exhibit slight differences in glycosylation when compared to their mammalian counterparts. As shown in previous studies, however, these differences do not affect important IgG activities, including complement lysis activity and antibody-dependent cell-mediated cytotoxicity (Boyd et al., 1995).

Although the level of antibody generated in our studies was lower than that resulting from the baculovirus expression system, our results clearly demonstrate that a biologically functional human antibody can be produced by transformed insect cells. The fact that a continuous and consistent supply of foreign protein can be generated with this system offers an advantage over baculovirus infection, for which expression is transient. If methods are developed for enhancing expression levels, the transformed insect cell expression system has the potential to be an attractive alternative to baculovirus or mammalian cell antibody expression.

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