Interference of Coronavirus Infection by Expression of Immunoglobulin G (IgG) or IgA Virus-Neutralizing Antibodies

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Immunoglobulin gene fragments encoding the variable modules of the heavy and light chains of a transmissible gastroenteritis coronavirus (TGEV)-neutralizing monoclonal antibody (MAb) have been cloned and sequenced. The selected MAB recognizes a highly conserved viral epitope and does not lead to the selection of neutralization escape mutants. The sequences of MAB 6A.C3 kappa and gamma 1 modules were identified as subgroup V and subgroup IIIC, respectively. The chimeric immunoglobulin genes encoding the variable modules from the murine MAb and constant modules of human gamma 1 and kappa chains were constructed by reverse transcriptase PCR. Chimeric immunoglobulins were stably or transiently expressed in murine myelomas or COS cells, respectively. The secreted recombinant antibodies had radioimmunoassay titers (i.e., the highest dilution giving a threefold increase over the background) higher than 10^5 and reduced the infectious virus more than 10^4-fold. Recombinant dimeric immunoglobulin A (IgA) showed a 50-fold enhanced neutralization of TGEV relative to a recombinant monomeric IgG1 which contained the identical antigen binding site. Stably transformed epithelial cell lines which expressed either recombinant IgG or IgA TGEV-neutralizing antibodies reduced virus production by >10^5-fold after infection with homologous virus, although a residual level of virus production (<10^2 PFU/ml) remained in less than 0.1% of the cells. This low-level persistent infection was shown not to be due to the selection of neutralization escape mutants. The implications of these findings for somatic gene therapy with recombinant antibodies are discussed.

The mucosal immune system and its predominant effector, secretory immunoglobulin A (IgA), provide the initial immunologic barriers against most pathogens that invade the body at a mucosal surface (22, 26, 30, 31, 40). This is especially true for viruses, since resistance to infection has been strongly correlated with the presence of specific IgA antibodies in mucosal secretions (2, 23). Traditionally, the neutralization of viruses by immunoglobulins is thought to result from the binding of antibodies to virion attachment proteins, thereby preventing adherence to epithelial cells. In addition, mucosal antibodies interact intracellularly with viruses, preventing their replication, possibly by interfering with virus assembly (13, 19, 26–28).

Conventional approaches such as lactogenic immunity and artificial feeding may target the antibodies to epithelial surfaces, providing protection against enteric virus infections (10, 35, 41, 49). New strategies, including the introduction of antibody genes into cells, have been recently explored in model tissue culture systems. Sindbis virus transient-expression vector has been used to deliver single-chain variable antibody fragment (Fv) genes into vertebrate cells, leading to protection against virus infection (19). This strategy may potentially be applied to in vivo protection of mucosal surfaces by gene therapy with antibody-encoding genes since monoclonal antibodies (MAbs) against a vast range of viruses are now available. Virus-neutralizing MAbs may protect mucosal tissues against viral infections; however it is not known whether antibody-secreting cells will provide protection to the neighboring tissues.

Transmissible gastroenteritis coronavirus (TGEV) infects both enteric and respiratory tissues and causes a mortality of close to 100% when newborn animals are infected (14, 35). The immune response to this virus has been characterized (1, 5, 46), and full protection against TGEV can be provided by lactogenic immunity from immune sows (10, 35, 41, 49). It has also been shown that the oral administration of serum elicited by recombinant adenoviruses expressing the spike protein completely protects pigs against virulent virus challenge (44, 45).

The major antigenic sites of TGEV involved in the induction of virus-neutralizing antibodies are located in the globular portion of the spike (S) protein (8, 11, 17). Investigations by our laboratory into the mechanisms of TGEV neutralization (42) and of antigenic and genetic variability (36, 37) have led to the identification of a mouse MAb which neutralized all TGEV isolates tested and which also neutralized TGEV-related coronaviruses that infect at least three animal species: pigs, dogs, and cats. This MAb probably binds to an epitope essential for virus replication since no neutralization escape mutants (nsar mutants) appeared when this MAb was employed (17).

We have studied the protection of epithelial cell monolayers against TGEV infection by using expression plasmids encoding virus-neutralizing MAbs. In this paper we describe the engineering of a recombinant TGEV-neutralizing MAb with an IgG1 isotype and the comparison of its protective activity with that of a recombinant dimeric antibody having identical variable modules and an IgA isotype. We found that cell lines stably transformed with these MAb-producing vectors were substantially protected against TGEV challenge; however, a small fraction of these cells continued to produce low levels of viral progeny. The implications of these findings with respect to
the possible efficacy of recombinant Mab (rMab)-mediated gene therapy are discussed.

MATERIALS AND METHODS

Cells and viruses. Swine testis (ST) (29) and simian virus 40 (SV-40)-transformed monkey kidney COS-1 cells (ATCC CRL-1650), nonsecreting murine myeloma Sp2/0 (ATCC CRL-1581) cells, and Mab 6A.C3- and Mab 1G7-secreting hybridoma (9, 20) were grown in Dulbeco’s modified Eagle medium supplemented with fetal calf serum. TGEV PUR46-MAD strain (17) was grown, purified, and titrated in ST cells as described previously (20). Vascular stomatitis virus (VSV) was grown and titrated as described previously (6).

Immunofluorescence microscopy. SV-40 and ST cells expressing recombinant mouse-human (rMH) antibodies, recombinant mouse-swine (rMS) antibodies, and the Mab 6A.C3-secreting hybridoma were grown in microculture chambers (Miles Scientific) at a density of 1.5 × 104 cells per cm². Cells were washed with phosphate-buffered saline (PBS) and fixed with methanol-acetone (1:1) at −20°C for 15 min. Cells were washed three times with PBS and once with 1% bovine serum albumin (BSA) in PBS for 30 min at room temperature. All antibody dilutions used in the double immunofluorescence were performed in PBS containing 0.3% BSA (PBS-BSA) (4). ST cells expressing rMH antibodies were incubated with rhodamine-labeled sheep anti-human IgG diluted 1:100, then with fluorescein-labeled mouse anti-human IgM (Cappel Laboratories) diluted 1:1,000 and then with fluorescein-labeled rabbit anti-goat antibody diluted 1:1,000. Infected cells expressing rMH antibodies were incubated with TGEV-specific polyclonal rabbit antiserum, then with fluorescein-labeled sheep anti-rabbit IgG (Amersham) diluted 1:1,000, and finally with rhodamine-labeled goat anti-rabbit IgG (Amersham) diluted 1:1,000 and with goat anti-swine IgA diluted 1:1,000. ST cells expressing rMH and rMS antibodies and untransformed ST cells were infected with TGEV at a multiplicity of infection (MOI) of 0.5. At 48 h postinfection (p.i.), cell monolayers were washed and fixed as described above. To detect recombinant antibodies and viral proteins in the same cells, double immunofluorescence was performed. Infected cells expressing rMH antibodies were incubated with rhodamine-labeled sheep anti-rabbit IgG and then with fluorescein-labeled mouse anti-human IgG diluted 1:1,000, and finally with rhodamine-labeled goat anti-rabbit IgG (Amersham) diluted 1:1,000.

RIA, virus neutralization, and Western blot analysis. The procedures for radioimmunoassay (RIA), virus neutralization, and Western blotting have been described previously (9). All antisera were diluted 1:1,000 in PBS-BSA and 0.1% Tween 20. The antisera used to develop the RIA assays to detect rMH antibodies were incubated with TGEV-specific polyclonal rabbit antiserum, then with fluorescein-labeled sheep anti-rabbit IgG (Amersham) diluted 1:1,000, and then with rhodamine-labeled mouse anti-human IgG (Cappel). To detect rMS immunoglobulins, rabbit anti-swine IgA (Bethyl, Montgomery, Tex.) was used. To detect Mab 6A.C3, rabbit anti-mouse immunoglobulin was used.

To compare the neutralizing activity of rMH IgG with that of rMS IgA, the same antisera (goat anti-mouse Fab 2) was used. To detect recombinant antibodies and viral proteins in the same cells, double immunofluorescence was performed. Infected cells expressing rMH antibodies were incubated with rhodamine-labeled sheep anti-human IgG diluted 1:100, then with fluorescein-labeled mouse anti-human IgM (Cappel Laboratories) diluted 1:1,000 and then with fluorescein-labeled rabbit anti-goat antibody diluted 1:1,000.

FIG. 1. Cloning of immunoglobulin L- and H-chain cDNAs into expression vectors. (A) Cloning of the rMH L-chain cDNA, PolyA+ RNA from hybridoma cells secreting MAb 6A.C3 was used as the template for an RT-PCR to obtain the cDNA encoding the VL module. The 5′ end oligonucleotide corresponding to the RNA leader (L) and the 3′ end oligonucleotide complementary to the first mouse constant-module nucleotides contained SalI and ClaI restriction endonuclease sites, respectively, to facilitate the cloning of the RT-PCR-derived cDNA into pBluescript SK+. The ClaI restriction endonuclease site starts at nucleotide 405 of the L-chain gene. The human L-chain (CL) module cloned into the expression vector pING2016e-gpt was modified by PCR to introduce a ClaI restriction site into the 5′ end. The resulting VL and CL fragments were joined at the ClaI restriction site and cloned into the expression vector pING2016e-gpt* by using the SalI and ScaI sites, yielding plasmid pINLC6A. (B) Cloning of the rMH H-chain cDNA, PolyA+ RNA from the hybridoma secreting Mab 6A.C3 was used as the template for an RT-PCR to obtain the cDNA encoding the H-chain module. The 5′ end oligonucleotide corresponding to the RNA leader (L) and the 3′ end oligonucleotide complementary to the first mouse constant-module nucleotides contained BamHI and an ApaI site, respectively, to facilitate the cloning of the RT-PCR-derived cDNA into pBluescript SK+. The ApaI restriction endonuclease site starts at nucleotide 426 of the H-chain gene. The human H-chain constant modules (CH1, CH2, and CH3) included in vector pGMH6 were digested with ApaI-BamHI, and the resulting fragment was ligated with the BamHI-ApaI fragment (containing the V module) into the BamHI restriction site of plasmid pING2003e-neo, yielding plasmid pINHC6A. En, SV-40 enhancer; Pr, SV-40 promoter; Apa, PolyA sequence; Pa, SV-40 polyadenylation signal.
Salt-Cla I VL fragment was amplified by RT-PCR from mouse MAb 6A.C3 mRNA and cloned into Bluescript SK−. This fragment was subcloned into the Salt-Cla I site of expression plasmid pNG2016G-ept+. The resulting plasmid was designated pNL6C6A.

The chimeric mouse-human H-chain expression vector was engineered (Fig. 1B) by ligating the BamH I-Apel VH fragment amplified by RT-PCR from mouse MAb 6A.C3 mRNA to the Apal-BamH I fragment from the human IgG1 constant-heavy chain module isolated from plasmid pGMI6 (50) and cloning the resulting cDNA into expression plasmid pNL2803E-neo (24, 50) previously digested with BamH I. The resulting plasmid was designated pNH6C6A.

Transformation of Sp2/0 myeloma and ST cells with immunoglobulin gene expression plasmids. For the expression of rMh IgG1 antibodies, L-chain pNL6C6A (5 μg) and H-chain pNIH6C6A (5 μg) plasmids were cotransfected. To express rMh IgA antibodies, L-chain pNLS6C6A (5 μg) and H-chain pNIH6C6A (5 μg) plasmids, previously described (39), were used. Plasmids were linearized at unique restriction sites after 3 days of immunoglobulin genes (δghII for pNL6C6A and pNLS6C6A and AurII for pNH6C6A and pPNIH6C6A) and were cotransfected into 105 Sp2/0 or ST cells by electroporation (33). Cells were seeded in a M-24 well microplate at 4 × 104 per well. Transfectants were selected in the presence of G418 (0.8 mg/ml) (4). The supernatants from all wells were positive for TGEV-specific antibodies by RIA. Cells showing the highest expression level were cloned twice by limiting dilution.

Transient expression of immunoglobulin genes in COS-1 cells. COS-1 cells (8 × 105) were transfected with the Lipofectin method (GIBCO BRL) with 5 μg of circular DNA of the same expression vectors used in the stable transformation. Antibody levels were evaluated by RIA and neutralization in supernatants harvested at the indicated times posttransfection.

Interference of TGEV infection in ST cell lines expressing recombinant TGEV-neutralizing antibodies. Cloned and uncloned ST cell lines expressing recombinant TGEV-neutralizing antibodies with the IgG1 or IgA isotype were grown in M-24 wells. After extensive washing, cells were infected with TGEV (MOI, 0.5) at 4°C. After 1 h of virus adsorption, the inoculum was replaced with recombinant TGEV-neutralizing antibodies. Antibody levels were evaluated by RIA and neutralization in supernatants from infected and noninfected cells were analyzed at 1 to 6 days (p.i.). Infections with VSV were performed in parallel as a control.

Nucleotide sequence accession number. The sequences obtained in this study have been assigned EMBL accession no. V6H6AC3, AF001495, and VLA6AC3, AF001496.

RESULTS

Sequences of genes encoding V modules of a TGEV-neutralizing MAb. In order to protect cell monolayers from virus infection by using MAbs, it is convenient to use an antibody that has a high titer for virus neutralization, that recognizes an epitope present in all virus isolates, and that does not lead to the selection of neutralization escape mutants. These conditions were fulfilled by MAb 6A.C3 (17, 20).

The sequences of MAb 6A.C3 V modules were determined by sequencing polyclonal RNA obtained from hybridoma cell line 6A.C3 and cloned cDNAs generated by RT-PCR. Both procedures gave the same result. The typical L- (Fig. 2A) and H-chain (Fig. 2B) organization was identified. L and H-chains of MAb 6A.C3 V modules contain three complementary determining regions, CD1R1, CD1R2, and CD1R3, and four framework regions, FR1, FR2, FR3 and FR4. Kappa and gamma signal peptides were encoded by the first 60 and 57 nucleotides, respectively (Fig. 2). These sequences were compared with homologous immunoglobulin sequences of Kabat’s database (21). The sequences of MAb 6A.C3 L- and H-chain V-module genes showed a high degree of homology with kappa (99.4%) and gamma 1 (92.7%) sequences of subgroup V and subgroup IIIC immunoglobulin genes, respectively. The J regions of the L and H-chains belong to the Jx type.

Generation of rMAb 6A.C3. The construction of the recombinant antibodies required the fusion of VL or VH modules to constant modules, which necessitated the introduction of ClaI and ApeI restriction endonuclease sites, respectively, into the immunoglobulin genes (Fig. 1). The mutagenesis required to introduce the restriction endonuclease sites did not introduce amino acid changes.

Human kappa and gamma constant modules were flanked by the SV-40 early promoter and polyadenylation signals and were subcloned into plasmids pNL6C6A and pNIH6C6A, respectively, which carry the mouse immunoglobulin enhancer at the 5’ ends of the expression cassettes (Fig. 1). Sequencing confirmed that V and constant immunoglobulin modules were correctly joined.

The engineering of recombinant IgA (rIgA) with the same V modules as those of rIgG1 has been described previously (39).

Functional analysis of recombinant MAbs with gamma 1 and alpha isoatypes. To verify the functionality of 6A.C3 rMAbs with IgG1 or IgA isoatypes, COS-1 cells were transiently co-transfected with plasmids encoding chimeric H and L-chains. To study antibody oligomerization Western blot analysis was performed under nonreducing conditions and demonstrated that MAb 6A.C3 with the IgG1 isotype was monomeric (molecular mass, 150 kDa), while rIgA presented a dimeric structure of about 300 kDa (results not shown). Antibody expression levels ranged between 20 and 40 μg/ml in the supernatant of transformed cells after 48 h; these levels were comparable to the antibody levels produced in other cell systems (47, 48). The secreted chimeric immunoglobulins bound TGEV, as determined by RIA, with titers (i.e., the highest dilution giving a threefold increase over background) up to 103 and neutralized virus infectivity around 104-fold (neutralization indices [NIs], around 4) (Table 1).

Murine Sp2/0 myeloma cells, which do not secrete endogenous immunoglobulins, were stably transfected by electroporation with constructs encoding the chimeric H and L-chains. Cell transformation frequencies with genes encoding both the H and L immunoglobulin chains ranged between 103 to 104 in the different experiments. Binding of the rMAbs to TGEV was determined by RIA of the supernatants from clones secreting the highest levels of antibodies. Titers obtained by RIA were similar to those obtained by transient transfection (Table 1).

The antibody H-chain frequently has a higher contribution to antigen binding than the L-chain. In fact, virus neutralization by purified antibody H-chains has been described previously (18). In the RIA, culture supernatants containing the rMAb 6A.C3 H-chain alone did not bind the virus or neutralize its infectivity, indicating that either the H-chain was not secreted efficiently or the contribution of the MAb 6A.C3 L-chain to TGEV binding must be essential.

The final aim of this work is to express the rMAbs in the swine enteric tract to examine their protective effect on mucosal surfaces against viral challenge. In these experiments IgA isotype antibodies are known to be more stable than those with the IgG isotype (22). To compare the neutralizing activities of rIgG1 and rIgA, supernatants containing recombinant antibodies at the same titer, as determined by RIA, were used in neutralization assays. Recombinant IgA neutralized TGEV 50-fold more effectively than rIgG1, as expected for a dimeric immunoglobulin (Fig. 3).

Generation of ST cells expressing rIgG1 and rIgA and evaluation of their resistance to TGEV infection. Porcine epithelial ST cells susceptible to infection with TGEV were transfected with DNA constructs encoding the chimeric H and L-chains to produce either the rIgG1 or the rIgA TGEV-specific antibodies. Twenty-four stably transformed cell lines expressing the recombinant MAb with each isotype were selected. Titers of the recombinant MAbs in supernatants from the cell lines obtained by RIA ranged from 102 to 103. Immunofluorescence microscopy studies using immunoglobulin-specific antibodies revealed that, before the stably transformed cells were cloned, 10 to 15% of the cells of each line expressed the recombinant MAbs (results not shown).

The 48 ST cell lines expressing rIgG1 or rIgA TGEV-specific antibodies were infected with TGEV (MOI, 0.5). Transformed cell lines were apparently resistant to TGEV infection (Fig.
4A) since no cytopathic effect was observed 48 h p.i., while untransformed ST cell monolayers were completely lysed. TGEV-infected ST cells produced 10^7 PFU/ml, while IgG1- and IgA-transformed ST cell supernatants had titers which dropped to 10^3 PFU/ml (Fig. 4B). This inhibition in viral synthesis was specific, since VSV grew to the same titer in transformed and untransformed cells (Fig. 4B). While there was a significant (10^4-fold) reduction in virus synthesis in the antibody-producing cells, they were not fully resistant to TGEV infection since a residual level of virus synthesis (10^3 PFU/ml) persisted in the absence of any discernible cytopathic effect.

Interference of TGEV infection in cloned ST cell lines expressing recombinant antibodies. To determine whether the residual infection of transformed ST cell lines by TGEV was due to the presence of a large proportion of cells that did not produce the antibody, two cell lines producing the highest body-producing cells, they were not fully resistant to TGEV infection since a residual level of virus synthesis (10^3 PFU/ml) persisted in the absence of any discernible cytopathic effect.
levels of either rlgG1 or rlgA antibodies were cloned by limiting dilution and infected (MOI, 0.5). All cloned cells expressed the expected rMAbs, either rlgG1 or rlgA, as determined by immunofluorescence microscopy (Fig. 5a and c), and 99.9% were free of viral antigens (Fig. 5b and d). No cytopathic effect was observed at 72 h p.i. in the transformed cells, in contrast to the lysis observed in the untransformed ST cell monolayer, which was similar to what was observed (Fig. 4) in the uncloned cells (results not shown).

The kinetics of antibody production, viral synthesis, and cytopathic effects were determined in rlgG1- and rlgA-transformed and untransformed cloned cell lines (Fig. 6). Virus production in the supernatants of transformed cultures was reduced from approximately $10^{7.5}$ to $<10^2$ PFU/ml. Cytopathic effects were not detected in rlgG-producing cells (Fig. 6C), and only minor effects were observed at 72 h p.i. in rlgA-producing cells. Nevertheless, when IgA-producing cells were passed the new cell monolayers reached confluence. A large reduction in the virus titer was detected ($>10^5$-fold), but a residual infectivity ($<10^2$ PFU/ml) persisted in the supernatants. Double immunofluorescence labeling showed the presence of viral antigens in a minor fraction ($<0.1\%$) of the antibody-producing cells (data not shown).

Low levels of infectious virus particles were produced by the transformed ST cells in the presence of virus-neutralizing antibodies (Fig. 6A and B). One possible explanation for these findings is that a neutralization-resistant virus could have been selected for. To test this possibility, the rMAb-producing ST cells were infected with TGEV (MOI, 0.5), the virus produced...
at 1, 2, 3, and 6 days p.i. was harvested, and neutralization by MAb 6A.C3 and by MAb 1G.A7, which recognizes a different antigenic subsite of the spike protein, was studied. Both MAbs were equally effective at neutralizing TGEV collected at different days p.i. (results not shown). Furthermore, MAb 6A.C3 completely neutralized the virus collected at the different time points. These results demonstrate that a neutralization escape mutant was not selected.

**DISCUSSION**

The genetic engineering and the anti-viral efficacies of two TGEV-neutralizing MAbs with the same binding site and IgG1 and IgA isotypes are described. The dimeric rMAb with the IgA isotype had a 50-fold higher efficiency in virus neutralization than the monomeric IgG1. The transformation of ST cells with plasmids encoding TGEV-neutralizing rMAbs with the IgG1 or IgA isotype reduced virus infectivity >10^5-fold and prevented the appearance of cytopathic effects. A low level of virus production was detected in a few antibody-producing cells.

**TGEV-neutralizing recombinant antibodies.** The rMH antibodies secreted by the immunoglobulin gene-transformed ST cells had titers, as determined by RIA, that were higher than 10^5 and that are comparable to those of the original hybridoma and NIs >3, indicating that a high level of functional chimeric antibodies was produced. Similar antibody production levels were achieved in myeloma (Sp2/0) and in epithelial (COS) cells, indicating that, in principle, the DNA constructs could be used to transform epithelial cells at mucosal surfaces for somatic gene therapy.

The specific activity of rIgA in TGEV neutralization was about 50-fold higher than that of monomeric rIgG1 with the same virus binding site. This was probably due to a combination of the higher avidity of the dimeric rIgA and to a higher steric hindrance of recombinant dimeric IgA in comparison with the monomeric rIgG1. In the influenza virus system, polymeric IgA neutralizes influenza virus to a larger extent than IgG (12, 32). Evidence has been presented (43) indicating that immunoglobulin polymerization affects the mechanism of neutralization. The larger polymeric structure of IgA-viral complexes may inhibit the endocytosis of attached neutralized virus by preventing the formation of a critical number of cell receptor-virus attachment site interactions needed for internalization.

**Protection of antibody-producing ST cell monolayers against virus infection.** A highly significant (>10^5-fold) reduction in virus recovery in rIgG- or rIgA-producing cell lines has been demonstrated. This reduction in virus production may suffice in vivo to provide protection against virus infection, in collaboration with other antiviral mechanisms, including nonspecific immune system effector molecules such as interferon and cellular immune responses that could cope with the residual virus or the virus-producing cells.

ST cell lines expressing a TGEV-neutralizing rMAb, in which only 10 to 15% of the cells were transformed, were
largely protected against TGEV infection. Nevertheless, protection was not complete, because low, but significant, levels of infectious TGEV were produced.

Supernatants of transformed ST cell clones had virus titers that were $<10^2$ PFU/ml, very low in comparison with the $10^7$ PFU/ml observed in untransformed cells under the same conditions. The decrease in virus titer probably was not due to a nonspecific reduction in the capacity of the transformed cells to produce virus, since the transformed ST cells produced VSV as efficiently as the untransformed cells. In addition, the TGEV titers were more similar in the supernatants of cell lines in which only 10 to 15% of the cells were transformed than in the cloned transformed cell lines in which 100% of the cells were antibody producers. The most likely explanation for this residual virus persistence is that TGEV neutralization is reversible and at the antibody concentrations present in the supernatants of the rMAb-transformed cells a small virus fraction ($10^2$ to $10^3$ PFU/ml) remains as free infectious virus while most of the virus ($10^4$ to $10^5$ PFU/ml) is in the form of noninfectious virus-antibody complexes.

Three mechanisms may be responsible for the reduction in virus production: (i) extracellular neutralization, (ii) intracellular neutralization, and (iii) modulation of virus production by antibodies (16). Extracellular neutralization is likely to be responsible for the results of this study, because the antibodies are continuously released into the medium, even during virus infection, and in the immunoglobulin gene-transformed uncloned cells about 85 to 90% of the cells did not produce the antibodies yet were still protected from TGEV infection.

Intracellular neutralization of virus in cultured cells has been demonstrated in the influenza and Sendai virus systems during transcytosis of dimeric IgA from the basal to the apical side of polarized epithelial cells (26). This mechanism may also operate in antibody-producing ST cells infected with TGEV, since during virus and antibody synthesis these proteins could colocalize, leading to an intracellular interference of protein transport as described previously for other systems (3, 7, 13, 25, 27, 34). It is not known whether intracellular neutralization is a mechanism that also operates in vivo.

Modulation of virus production by antibody binding to viral antigens on the surface of virus-infected cells is a phenomenon that operates in several virus systems (16). This mechanism may modulate TGEV synthesis in antibody-producing ST cells. This modulation of virus synthesis probably leads to the establishment of a persistent infection, since residual virus production was often observed for at least 3 weeks p.i. (results not shown). Antibody-induced modulation is a mechanism by which other viruses can persist and escape immunologic surveillance (16). Experimental evidence has suggested such a mechanism for measles virus, herpes simplex virus, and retrovirus.

The majority of the virus produced by the transformed cells was probably identical to the original one used for infection. A neutralization escape mutant was not selected for because the virus produced at days 1 and 6 p.i. was neutralized with the same efficiency by MAb 6A.C3, produced by the transformed ST cells, and by MAb 1G.A7, which binds to a different antigenic subsite of the TGEV spike protein (9, 17). This is in agreement with our previous results indicating that the epitope recognized by MAb 6A.C3 is an interspecies conserved epitope, present in porcine, canine, and feline coronaviruses, which seems to be essential for virus replication, and for which neutralization escape mutants have never been observed (37).

The transient expression of virus-neutralizing antibodies in mucosal surfaces could be used to provide immediate protection in the mucosal areas. This type of somatic gene therapy could be of potential benefit to protect nonvaccinated individuals such as newborn animals. In addition, this type of therapy could be used to prevent the allergic immune responses produced by the predominance of an IgE isotype response by using recombinant virus vectors encoding antibodies with IgG isotypes.

The transformation of virus-susceptible cells with genes encoding virus-neutralizing antibodies has led to a $>10^4$-fold reduction in virus recovery. Although some residual virus synthesis persists in less than 0.1% of the cells in culture, it is possible that in vivo defense mechanisms apart from virus neutralization, such as cytolytic T cells, could completely control virus infection. This new strategy may be particularly useful in infections where fast immune intervention is required in a defined tissue, since viral vectors could express antibody genes within 2 to 3 h and since this expression may be targeted to specific tissues.

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