62

OVEREXPRESSION OF TGEV CELL RECEPTOR IMPAIRS THE PRODUCTION OF VIRUS PARTICLES

B. Delmas, E. Kut, J.Gelfi, and H. Laude

Unite de Virologie et Immunologie Moléculaires
INRA
Jouy-en-Josas, France

ABSTRACT

The porcine aminopeptidase-N (pAPN) is the cellular receptor for the transmissible gastroenteritis virus (TGEV) due to the specific binding of the spike protein S to APN. In the present study, we performed both biological and biochemical experiments to analyze how the level of expression of a virus receptor can influence the viral protein biosynthesis and the virus production. We generated two swine testis cell clones overexpressing pAPN (ST-APN clones). These clones produced 10^4 less infectious virus than control ST cells. Plaque assays revealed a four-fold reduction of the diameter of the plaques in ST-APN cells compared to ST cells. Pulse-chase experiments revealed that S transport from the endoplasmic reticulum to the Golgi apparatus was not affected in ST-APN cells. Additionally, an anti-APN antibody was able to increase the virus released in the supernatant of ST-APN cells. Likewise, BHK clones expressing variable amounts of pAPN were shown to acquire TGEV susceptibility and to produce infectious particles as an inverse function of their level of pAPN expression. In contrast, MDCK clones expressing low or large amounts of pAPN failed to produce infectious particles. Taken together, these studies strongly suggest that overexpression of receptor, but also other(s) undetermined factor(s), can impair the production of viral particles.

INTRODUCTION

Aminopeptidase N (APN) has been shown to be the receptor of the porcine transmissible gastroenteritis virus on target cells by the following main criteria: i) antibodies specific of pAPN blocked efficiently the entry of TGEV in permissive cells; ii) a physical interaction between TGE virions or the spike protein S and pAPN has been evidenced, iii) pAPN expression conferred susceptibility to TGEV to non permissive cells and iv) pAPN is cointernalized with virions during the early steps of infection[1,2,3]. These observations support the view that S-pAPN complex formation at the cell surface represents the first critical step

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

in the infection. However, little is known about the importance of the level of APN expression for the entry of virus or subsequent steps of the virus cycle. The swine testis ST cell line, which is highly susceptible to TGEV infection, provide a useful model to analyze how the level of expression of a virus receptor can influence the viral protein biosynthesis and the virus production. ST clones overexpressing pAPN were selected and were analyzed individually for S biosynthesis and capacity to produce virions. To extent the results, pAPN-expressing clones were derived from BHK and MDCK cells, two cell lines refractory to TGEV, and viral progeny was quantified as a fonction of the level of pAPN expression.

MATERIALS AND METHODS.

Virus and cell transfections. The high cell-passage Purdue-115 strain was used as a TGEV source. The cDNA encoding the pAPN was subcloned in the pTEJ4 expression vector[1]. ST, BHK-21 and MDCK cells were cotransfected with this construct and pSV2neo by lipofection or by $CaPO_4$ (three MDCK clones[1]). Cell clones resistant to the neomycin analogue G418 were selected and assayed for APN expression and TGEV susceptibility acquisition (all clones except ST clones which are naturally TGEV susceptible). APN activity was quantified as described[1].

Radiolabeling and zonal centrifugation of viruses. Confluent cell monolayers in 6 wells Costar plates were labeled 4h post-infection for 5-7hrs with 50 mCi of Tran^{35}S-label (ICN) in Eagle's MEM supplemented with 5%CS. The medium was clarified and layered onto 20 to 45% (w/w) sucrose/water gradient in SW40 rotor (Beckman). After centrifugation for 4h at 25,000 r.p.m., 0.5 ml aliquots were collected and viral antigens revealed by immunoprecipitation using a mixture of anti-S, -M and -N monoclonal antibodies as described[4].

Pulse-chase labeling. ST and ST-APN cells in 12 wells Costar plates were labeled at 6.5 h p.i. for 15 min with 120 mCi Tran^{35}S-label. After a period (0 to 60 min) of incubation in an excess of non-radioactive methionine and cystine, cells were solubilized by adding 0.3 ml of lysis buffer.

RESULTS

Negative Correlation Between the Level of APN Expression and the Virus Production in ST Cell Clones

ST cells were transfected with pAPN-cDNA to obtain two ST-APN clones expressing 10 to 50 fold higher amount of APN than control ST clones. Table 1 shows that ST-APN clones produced approximately 10^4 less virus than ST clones transfected only with the plasmid encoding the antibiotic resistance. To confirm this observation, plaque assays were realized in parallel with ST and ST-APN cells. We observed a five fold increase of the plaquing efficiency in APN-overexpressing cells, probably due to a higher adsorption of virions, but concomitantly a four fold reduction of the diameter of the plaques (not shown). These results suggest that overexpression of APN partially impairs the virus production. To extend this observation, we analyzed virus production by cell radioactive labeling and rate zonal centrifugation of virus particles released in the supernatant (Fig.1). Virus particles were consistently found in lower amount in ST-APN cells supernatant compared to that in ST cells. As quantified by autoradiography scanning (and in these experimental conditions), ST-APN clones produced 10 to 50 less virus than in ST cells. The relative proportion of the three main structural proteins S, M and N did not appear to be modified in virions produced in ST-APN cells.

Table 1. TGEV infectious virus production in ST and ST-APN cell clones

Cell line	Virus production[a] (PFU/ml)
ST cells clone 2	1.3×10^8
ST cells clone 3	3.9×10^8
ST cells clone 7	4.9×10^8
ST APN cells clone 2	$<5.0 \times 10^4$
SR APN cells clone 11	$<5.0 \times 10^4$

[a]Total infectivity titer was determined 20 h post infection by plaque assay on ST cells

S Maturation in ST-APN Cells

A possible explanation for the down-production of virus in ST-APN cells is that intracellular APN affects the biosynthesis of the S protein The synthesis of viral antigens was analyzed by cell radioactive labeling and immunoprecipitation Similar amounts of neosynthesized structural proteins were observed in ST and ST-APN cells (not shown) Moreover, the rate of S conversion from the 175K band (Endo H sensitive form of S) into the 220K band (Endo H resistant)[5] was not modified in the ST-APN cells (Fig 2)

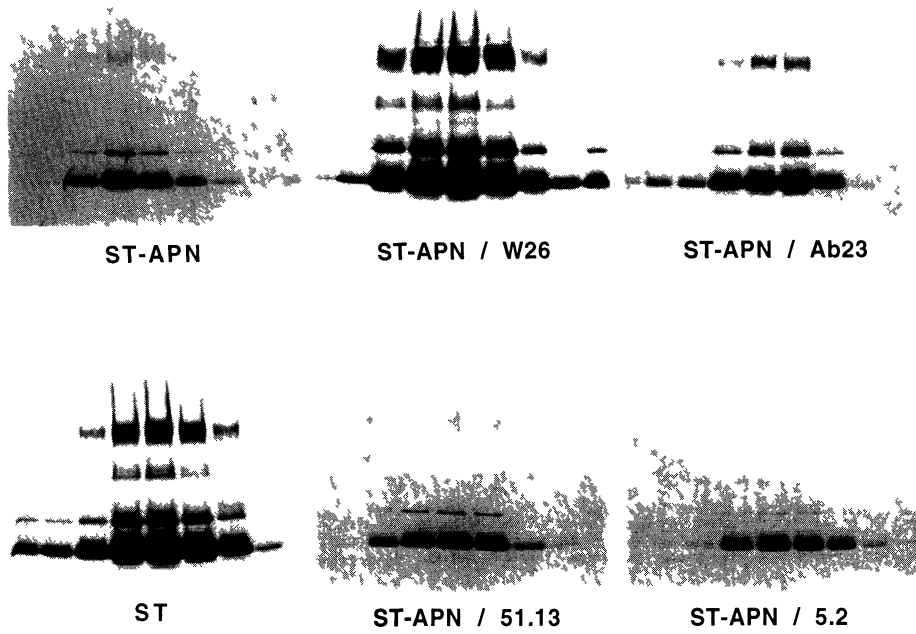

Figure 1. Virus production in ST and ST APN cells Five hours p i cells were incubated or mock incubated with the indicated antibodies W26 Ab23 (anti APN) or 51 13 5 2 (anti S) Nine hours p i cell supernatants were fractionated by rate zonal centrifugation Even fractions were immunoprecipitated with anti S N and M antibodies Viral polypeptides were visualized by 8 to 15% polyacrylamide gradient SDS-PAGE and autoradiography

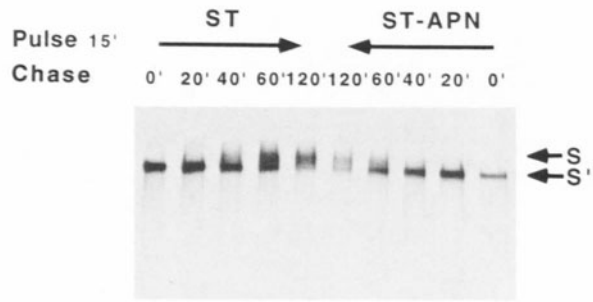

Figure 2. TGEV S protein maturation in ST and ST-APN cells. Cells were radiolabeled for 15 min, then chased 0 to 120 min. Immunoprecipitates with antibody 51.13 were resolved on an autoradiographed 8% SDS-PAGE.

An Anti-APN Antibody Restores Virus Production in ST-APN Cells

To determine if virus down-production in ST-APN cells could involve a late event in the virus maturation, we incubated infected cells with an anti-receptor antibody four hours p.i. The anti-APN antibody bound to APN is expected to be endocytosed and to reach intracellular vacuoles[6]. Interestingly, this resulted in an increased virus amount up to a level comparable to that in normal ST cells (Fig. 1). This effect was observed at concentrations of antibody as low as 1.25 μg/ml (not shown). Two anti-spike antibodies were used as controls, with one of them (51.13) having a strong neutralizing activity[4]. None of these antibodies was found to increase the amount of virus present in the cell supernatant.

Level of APN Expression and Virus Production in pAPN-BHK Cell Clones

Seven clones derived from the BHK-21 cells and expressing variable amount of recombinant pAPN were selected. All of them were susceptible to TGEV as shown by measurement of the cytopathic effect induced by the infection. Fig. 3 shows that four

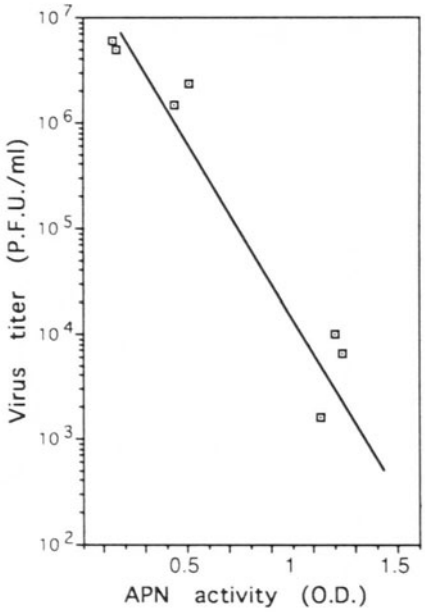

Figure 3. TGEV infectious virus production in seven BHK pAPN-expressing clones. Total infectivity titer was determined 20h p.i. by plaque assay on ST cells. APN activity was quantified by cleavage of the chromogenic substrate leucine *p*-nitroanilide.

Table 2. TGEV infectious virus production in MDCK cell clones

Cell line	APN activity (uU/ml)	Virus production[a] (PFU/ml)
MDCK-APN cl 1	158 3	5
MDCK-APN cl 2	162 5	160
MDCK-APN cl 3	128 2	35
MDCK-APN cl 12	3 5	$1\,6 \times 10^3$
MDCK-APN cl 21	3 7	$5\,9 \times 10^2$
MDCK	3 2	$4\,6 \times 10^3$
ST	2 0	5×10^7

[a]Total infectivity titer was determined 20 h post infection by plaque assay on ST cells

BHK-derived clones expressing low amount of pAPN produced infectious virions in the same range as the highly permissive swine testis cell line ST ($1x10^6$ to $6x10^6$ p f u /ml) In contrast, the three BHK clones expressing high amount of pAPN produced few amount of particles Thus, like in ST cells, we observed an inverse correlation between the capacity of the clones to produce infectious virus and their level of pAPN expression

MDCK Cells Are Refractory to TGEV Virion Production

In a earlier study, we showed that three MDCK derived clones expressing large quantities of pAPN failed to produce virus[1] To see whether it was due to the level of APN expression, two MDCK clones transfected with pAPN-cDNA were selected on both their acquired susceptibility to TGEV by measurement of the c p e induced by the infection and on an APN activity of the same range than the parental MDCK cell line Titration of infectious viral progeny produced in the five MDCK clones shown that all of them failed to produce infectious particles compared to ST cells (Table 2) The low titer observed with the MDCK clones overexpressing pAPN was probably due to a better endocytosis and uncoating of the infectious virus, thus lowering the titer of the residual input virus This observation indicates that, in contrast to that found in ST and BHK cells, the impaired virus production is not related to an overexpression of APN

DISCUSSION

In the present study, we investigated the correlation between the level of receptor expression and the production of TGE virions in different cell systems As a main result, we observed a strong negative correlation between the level of pAPN receptor expression and the ability of the cell to produce virus particles in two different cell lines the swine testis cell line ST, naturally susceptible to TGEV and the baby hamster kidney cell line BHK, susceptible after pAPN-cDNA transfection A 10,000-fold reduction of the virus production was observed in ST clones overexpressing APN compared to that in non-transfected clones Similar observations were made with BHK clones It was also observed that the virus plaque formed in ST-APN cells were reduced in size, a finding which corroborates the decreased infectious virus production Concomitantly, the efficiency of plaquing was found to be significantly enhanced in these cells, thus indicating that the amount of receptor on the cell surface is a limiting parameter in the viral adsorption A series of experiments were carried out in an attempt to identify the step of the virus cycle potentially affected in APN-overex-

pressing cells. First, we tried to detect an intracellular interaction between the S spike and pAPN in pAPN-overexpressing clones without obtaining clear evidence for this (not shown). Moreover, pulse chase experiments showed that S transport (conversion from a endoH sensitive to a endoH resistant form) was identical in ST-APN and in ST cells (half time, 40mn). This suggests that, if there is a binding between APN and S inside the endoplasmic reticulum or the Golgi apparatus, it does not interfere with the spike maturation. Another possibility was that APN bound to S later in the virus cycle, during the budding or the release of the virus from the cellular membranes. The fact that the addition of an anti-pAPN antibody is able to restore a virion production nearly identical to that obtained with ST cells showed that the defectiveness of ST-APN cells to produce virion involved a late event in the virus replication cycle, such as a APN-S binding in virus secretion vesicles. To explore this hypothesis, we plan to compare the virus maturation in ST and ST-APN cells by electron microscopy.

The above observations raise the question of how TGEV can replicate and propagate efficiently in the natural target cells, the enterocytes[7], which are known to express APN at a level equivalent, if not higher, than the overexpressing cell lines here studied. An attractive explanation is that a productive infection in vivo involves a virus-driven down-regulation of the pool of receptor molecules, as recently evidenced for other viruses[8,9].

A third cell line, MDCK, was investigated in the present study. At difference with the ST and the BHK cells, MDCK cell clones expressing APN at a low level were still deficient for infectious virus production. As a confirmation, no plaque formation could be obtained in APN-MDCK cells. Nevertheless, as previously reported, infection of the cells at a high m.o.i. led to a strong cytopathic effect as well as to viral antigen synthesis[1]. Thus, the virus production is unlikely to involve an early step of the virus cycle. Such an abortive replication clearly differs of the situation reported for human APN-expressing MDCK clones infected with HCV-229E[10] or for MDCK cells transfected with feline calicivirus genomic RNA[11], where no viral synthesis could be detected. Elucidating which step of TGEV replication is severely impaired in pAPN-expressing MDCK cells might contribute to a better understanding of the coronavirus assembly or budding processes.

REFERENCES

1 Delmas, B , Gelfi, J , L'Haridon, R , Vogel, L K , Sjostrom, H , Noren, O , Laude, H Aminopeptidase N is a major receptor for the enteropathogenic coronavirus TGEV Nature 1992,357 417-420

2 Godet, M , Grosclaude, J , Delmas, B , Laude, H Major receptor-binding and neutralization determinants are located within the same domain of the coronavirus transmissible gastroenteritis virus spike protein J Virol 1994,68 (in press)

3 Hansen, G , Vogel, L K , Delmas, B , Gelfi, J , Laude, H , Noren, O , Sjostrom, H The coronavirus TGEV causes infection after endocytosis by an aminopeptidase N recruiting mechanism (manuscript in preparation)

4 Laude, H , Chapsal, J -M , Gelfi, J , Labiau, S , Grosclaude, J Antigenic structure of transmissible gastroenteritis virus I Properties of monoclonal antibodies directed against virion proteins 1986,67 119-130

5 Delmas, B , Laude, H Assembly of coronavirus spike protein into trimers and its role in epitope expression J Virol 1990,64 5367-5375

6 Louvard, D Apical membrane aminopeptidase appears at site of cell-cell contact in cultured kidney epithelial cells Proc Natl Acad Sci USA 1980,77 4132-4136

7 Pensaert, M , Callebaut, P , Cox, E Enteric coronaviruses of animals In Kapikian A Z , (ed) Viral infections of the gastrointestinal tract 2nd ed Marcel Dekker, Inc , New York 1993 pp 627-696

8 Bour, S , Geleziunas, R , Wainberg, M A The role of CD4 and its downmodulation in establisment and maintenance of HIV-1 infection Immunological Reviews 1994,140 147-171

9 Naniche, D , Wild, T F , Rabourdin-Combe, C , Gerlier, D Measles virus haemagglutinin induces down-regulation of gp57/67, a molecule involved in virus binding J Gen Virol 1993,74 1073-1079

10 Delmas, B , Gelfi, J , Kut, E , Sjostrom, H , Noren, O , Laude, H Determinants essential for the transmissible gastroenteritis virus-receptor interaction reside within a domain of aminopeptidase-N that is distinct from the enzymatic site J Virol 1994,68 5216-5224
11 Kreutz, L C , Seal, B S , Mengeling, W L Early interaction of feline calicivirus with cells in culture Arch Virol 1994,136 19-34