Expression of bovine herpesvirus type 1 glycoprotein gI in transfected bovine cells induces spontaneous cell fusion

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Bovine MDBK cells were transfected with Rous sarcoma virus-based vectors for constitutive expression of the bovine herpesvirus type 1 (BHV-1) glycoprotein, gI. Cell lines stably expressing recombinant gI were cloned and characterized. Recombinant gI was localized intracellularly, predominantly in a perinuclear region, and on the cell surface. Cells expressing gI exhibited spontaneous polykaryon formation, thus confirming the fusogenic activity described previously in gI-expressing transfected murine LMTK- cells. The recombinant form of gI synthesized in transfected MDBK cells was similar in Mr to the form expressed in BHV-1-infected MDBK cells, unlike the recombinant form of gI expressed by LMTK- cells which is deficient in N-linked glycosylation. It was concluded that cell fusion associated with the expression of BHV-1 gI in transfected mammalian cells is a reproducible phenomenon in a number of cell types and is not due to species-specific factors or expression of abnormally glycosylated gI. Cell fusion is a useful in vitro marker for gI function and may contribute to the spread of BHV-1 infections in vivo.

Two expression vectors, pRSVgI and pRSDneogI, containing the gI-coding sequence under the control of the Rous sarcoma virus long terminal repeat enhancer-promoter were constructed as previously described (Fitzpatrick et al., 1988, 1990). The vector pRSDneogI contains all of the elements of pRSVgI but with the addition of the neomycin resistance gene, under the control of the simian virus (SV40) enhancer-early promoter. Plasmid DNA was manipulated using standard methods, purified by caesium chloride gradient centrifugation, and sterilized by ethanol precipitation before transfection (Maniatis et al., 1982). Bovine MDBK cells were grown in Eagle's MEM supplemented with 10% foetal bovine serum as previously described (Babiuk et al., 1975) and seeded at 50% confluence 24 h before transfection. Transfections were conducted using the calcium phosphate precipitation method followed by glycerol shock as described in detail by Fitzpatrick et al. (1988) but with two modifications: pSV2neo DNA was omitted from precipitates containing pRSDneogI DNA, and post-transfection sodium butyrate treatment was omitted. Transfected cells were selected for G418 resistance and stable cloned cell lines constitutively expressing recombinant gI were derived by limiting dilution. The following results were consistently obtained with eight clones from two independent transfections with each expression vector, as well as with two clones derived from transfection of a bovine viral...
diarrhoea virus, mycoplasma- and reverse transcriptase-free MDBK cell line (Bielefeldt Ohmann et al., 1987) with pRSDeogI.

Expression of recombinant gI was detected by immunocytochemistry using methanol-fixed and permeabilized cells, gI-specific monoclonal antibodies (MAb; van Drunen Littel-van den Hurk et al., 1984), and an avidin–biotin-enhanced immunoperoxidase staining method (Fitzpatrick et al., 1988). Recombinant gI was localized predominantly in a perinuclear region with some diffuse cytoplasmic staining and faint nuclear membrane staining (Fig. 1). Transfected MDBK cells expressing gI exhibited extensive polykaryocytosis with multinucleated cells typically containing three to six nuclei (Fig. 1). In contrast to the fusion phenomenon described in transfected LMTK− cells expressing gI (Fitzpatrick et al., 1988), little nuclear fusion or giant cell formation was observed. The frequency of polykaryons varied from approximately 8 to 20% for the 10 transfected cell clones noted above and was most readily detectable when the cells were 25 to 75% confluent. MDBK cells expressing BHV-1 gIII or transfected with pSV2neo alone did not stain for gI expression and did not exhibit polykaryocytosis (data not shown). Recombinant gI was also localized in the plasma membrane of transfected MDBK cells. This was not detectable by immunocytochemistry (data not shown) but was demonstrated using flow cytometric analysis (Fig. 2) of transfected cells labelled with a gI-specific MAb followed by a fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Fitzpatrick, 1989).

Comparative analysis of the forms of gI synthesized in BHV-1-infected MDBK cells, transfected MDBK cells and transfected LMTK− cells was conducted by radioimmunoprecipitation of gI from lysates of [35S]methionine-labelled cells, with a gI-specific MAb and rabbit anti-mouse IgG-coated Protein A–Sepharose beads, followed by SDS-PAGE separation of reduced, denatured samples (Fitzpatrick et al., 1988). These studies revealed that the recombinant form of gI expressed by transfected MDBK cells was completely processed proteolytically into two cleavage fragments with Mr values of 75K and 55K, which were identical in Mr to the equivalent fragments precipitated from BHV-1-infected MDBK cells (Fig. 3, lanes 2, 3 and 4). In contrast, the fragments precipitated from transfected LMTK− cells have Mr values of approximately 72K and 55K (Fig. 3, lane 5) due to deficient N-linked glycosylation of the 75K fragment (Fitzpatrick et al., 1988 and unpublished results). The forms of gI precipitated from BHV-1-infected MDBK cells included a small amount of the uncleaved 130K precursor, as well as the two mature cleavage fragments (Fig. 3, lane 3; van Drunen Littel-van den Hurk & Babiuk, 1986).
Fig. 2 Flow cytometry of transfected MDBK cells expressing BHV-1 gI. (a) pSV2neo-transfected MDBK cells; (b) pRSVgI- plus pSV2neo-transfected MDBK cells; (c) pRSDneo gI-transfected MDBK cells. Dashed lines indicate samples labelled with an unrelated MAb; solid lines indicate samples labelled with a gI-specific MAb. Monolayers of transfected cells were dispersed by treatment with versene containing 0-25% trypsin for 5 min at 37 °C, incubated in 2% normal goat serum for 30 min at 4 °C and labelled with a MAb for 1 h at 4 °C. The cells were washed and then labelled with fluorescein isothiocyanate-conjugated goat anti-mouse IgG for 30 min at 4 °C, washed again and then fixed with 2% formaldehyde for 16 to 24 h at 4 °C, and finally subjected to flow cytometric analysis with 10000 cells per sample (Fitzpatrick, 1989).

In addition to the biochemical similarity between the mature forms of gI expressed in transfected and infected MDBK cells, comparative analysis of the antigenic reactivities of these forms was done, by radioimmunoprecipitation and immunocytochemistry with a panel of MAbs and polyclonal rabbit and bovine antisera specific for gI (van Drunen Littel-van den Hurk & Babiuk, 1985). It revealed no differences in reactivity between the recombinant and native glycoproteins (data not shown).

Constitutive expression of recombinant gI in transfected MDBK cells was obtained using a strategy similar to that employed previously for LMTK- cells (Fitzpatrick et al., 1988), but using modified Rous sarcoma virus-based expression vectors which are more efficient than SV40-based vectors in transfected bovine cells (Fitzpatrick, 1989). Cloned cell lines derived from transfections with the vector pRSDneo gI consistently exhibited higher levels of gI expression than clones derived from pRSVgI transfections, both in MDBK cells (Fig. 2 and 3) and in LMTK- cells (data not shown).
of gI synthesized in BHV-l-infected bovine cells, in expression of gI in transfected MDBK cells was cells. The perinuclear accumulation and cell surface localization and structural properties resembled the distribution of gI described for BHV-1-infected MDBK cells at approximately 6 to 12 h post-infection (Okazaki et al., 1987). In addition, the recombinant form of gI expressed in transfected MDBK cells appeared to possess greater structural integrity in terms of its Mr, which closely resembled that of the form of gI synthesized in BHV-1-infected bovine cells, in contrast to the abnormal form expressed in transfected LMTK- cells (Fig. 1 and 2; Fitzpatrick et al., 1988). These results suggest, as previously hypothesized (Fitzpatrick et al., 1988), that three factors may affect the synthesis and processing of gI expressed in different cells. Firstly, cell-specific quantitative differences in glycoprotein synthesis, processing and/or transport pathways could affect the degree of perinuclear accumulation of gI. Secondly cell-specific qualitative differences in glycosylation pathways might result in synthesis of an apparently normally glycosylated form in transfected LMTK- cells and an atypical recombinant molecule in transfected LMTK- or infected MDBK cells. Thirdly, virus-inhibited cellular proteolytic cleavage processes may completely cleave gI into two fragments in the transfected MDBK and LMTK- cells (Fig. 3), but may cleave the 130K precursor molecule incompletely in BHV-1-infected cells, probably due to BHV-1-induced host cell shut-down (Fitzpatrick et al., 1988; van Drunen Littel-van den Hurk et al., 1989).

In spite of these differences, the functional and antigenic properties of the recombinant form of gI expressed in transfected MDBK cells were similar to those described previously for the recombinant form of gI expressed in transfected LMTK- cells (Fitzpatrick et al., 1988). It is particularly noteworthy that the fusogenic activity associated with gI expression, observed previously in transfected LMTK- cells (Fitzpatrick et al., 1988), was reproducible in transfected MDBK cells. This result suggests that gI-associated cell fusion is not species-specific, as recently suggested (Chase et al., 1989) and that it is not dependent on the abnormal intracellular distribution of an atypical recombinant molecule. The lack of cell fusion in gI-expressing, transfected bovine fibroblasts noted by Chase et al. (1989) may be due to a number of factors, including cell-specific differences between MDBK cells and bovine fibroblasts, use of an inducible rather than a constitutive expression system, different cell culture conditions during selection and cloning procedures, and/or quantitative differences in gI expression levels.

We conclude that cell fusion in gI-expressing transfected mammalian cells is a reproducible marker of gI function in a number of cell lines and may be a useful in vitro model system for dissection of the important structural domains of the molecule. The cell fusion phenomenon may also be relevant for BHV-1 infections in vivo; however, it is likely that in vivo expression of the fusogenic activity of gI is modified by a number of other virus- and host-associated factors which contribute to virus spread in infected animals.

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References


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