ROLE OF pH IN SYNCYTIUM INDUCTION AND GENOME UNCOATING OF AVIAN INFECTIOUS BRONCHITIS CORONAVIRUS (IBV)

D. Li and D. Cavanagh

AFRC Institute for Animal Health, Houghton Laboratory
Houghton, Huntingdon Cambridgeshire, PE17 2DA, UK

SUMMARY

Syncytium formation induced in Vero cells by IBV-Beaudette was optimal at pH 6.7 and absent at pH 7.5. Reduction of IBV-Beaudette replication by ammonium chloride, which raises the pH in endosomes, further indicated that uncoating of the virus genome probably occurred within the acidic environment of endosomes. However, this would not appear to apply to all IBV strains, since one was unaffected by the drug. It would appear that some strains of IBV have the capacity to fuse with the plasma membrane at neutral pH, thereby leading to genome uncoating at that site. Other strains of IBV might also uncoat at the plasma membrane when the extracellular environment is slightly less than pH7.

INTRODUCTION

The large spike (S) glycoprotein of coronaviruses is involved in several processes[1]: attachment of the virus to cells; fusion of the virus envelope with cell membranes resulting in uncoating of the genome; and fusion of the cell surface membrane of infected cells with that of neighbouring cells to form syncytia. The latter process is caused by the presence of S, synthesised following infection, at the cell surface and is referred to as fusion-from-within (FFWI). Whether genome uncoating of IBV occurs following fusion with the cell surface (plasma) membrane or with internal membranes eg. within the acidic environment of endosomes or lysosomes, is unknown. We have addressed this question.

MATERIALS AND METHODS

IBV strains. Beaudette-US (adapted to Vero cells)[2]; H120; D1466; D274; UK/123/82.

Cells. Vero and chick kidney (CK) cells were used. Maintenance medium for experiments was Eagle's minimal essential medium buffered with 20mM BES (N,N-bis[2-hydroxyethyl]-2-Aminoethane sulfonic acid) and various concentrations of sodium bicarbonate to achieve the desired pH.

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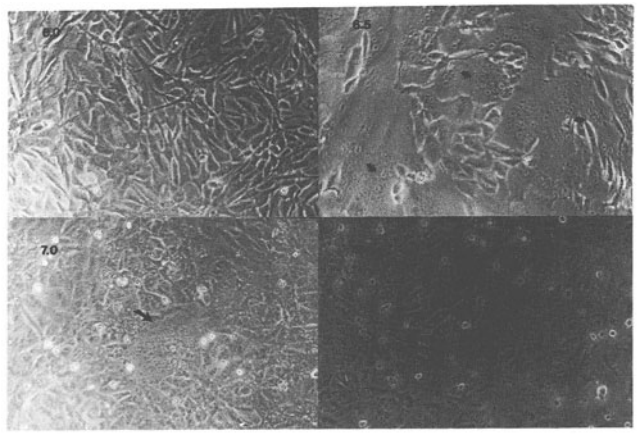

Fig.1. pH dependence of syncytium induction in Vero cells by IBV-Beaudette-US. Cells were infected at an MOI of 0.02 pfu/cell and incubated at the indicated pH for 36h at 38°C. Syncytia are indicated by arrows.

RESULTS

pH Requirement for IBV-induced cell-cell fusion. Vero cells inoculated with IBV-Beaudette-US (multiplicity of infection (MOI) 0.02 pfu/cell) were subsequently incubated at different pH. After 36h at 38°C there was extensive syncytium formation, resulting from FFWI, at pH 6.5 and 7.0 but none at pH 6.0 and 7.5 (Fig.1).

Analysis of infectious virus production and viral RNA synthesis (not shown), revealed that IBV replication was very low at pH 6.0 and 7.5. pH 6.0 itself was not inhibitory for membrane fusion. Thus cells inoculated at an MOI of 0.2pfu/cell and incubated at pH 7.5 did form syncytia when the infected cells were exposed to pH 6.0 for 30 min at 36h post inoculation (p.i.). Maximum fusion was obtained when cells were incubated at pH 6.7 (data not shown).

Table 1. Titre* of IBV-Beaudette-US grown in Vero cells in the presence of ammonium chloride

Multiplicity (pfu/cell)	hours p.i.	concentration of ammonium chloride (mM)				
		0	5	10	20	30
0.3	12	2.4	1.5	0	0	0
	24	4.7	3.3	2.0	2.0	0
	36	5.8	4.8	2.7	0	0

* Virus was titrated by plaque assay in Vero cells and is expressed as $\log_{10}$ pfu/ml.

Table 2. Effect of ammonium chloride on the early infection of IBV-Beaudette-US in Vero cells*

Groups	NH_4Cl 0-2 hours p.i.	NH_4Cl 2-4 hours p.i.	No of infected cells at 14 hours p.i.	No of infectious foci at 14 hours p.i.
1	+	-	10.5	2
2	-	-	138.5	38
3	-	+	180.0	31
4	-	-	171.5	35

* Vero cells were infected at a multiplicity of 0.3 pfu per cell. Infected cells and infectious foci were detected by immunofluorescence.

Location of genome uncoating. Ammonium chloride raises the pH in endosomes and would be expected to inhibit genome uncoating of a virus which required an acidic endosomal environment for uncoating. Vero cells were incubated for 30 min with ammonium chloride and then inoculated, in the presence of the drug, with Beaudette-US at 0.3 pfu/cell. After washing incubation was continued in the presence of ammonium chloride. Table 1 shows that infectious virus production was reduced by 10 to 1000-fold, depending on the concentration of the drug. Preliminary work had established that the Vero cells remained viable under the conditions used.

Reduction of virus production also resulted when ammonium chloride was present only for the first 2h after infection but not when the drug was added after 2h of incubation (Table 2). Several experiments showed that ammonium chloride had not (a) had a direct virucidal effect, (b) reduced virus attachment to cells or (c) greatly inhibited cell RNA synthesis. The inhibitory effect of ammonium chloride also occurred with 3 other strains of IBV, as assessed by immunofluorescence of infected CK cells. However, strain UK/123/82 was reproducibly not affected by ammonium chloride, suggesting that the drug did not have general effects on IBV translation, transcription, protein processing and transportation and virus maturation. Thus, the replication of IBV-Beaudette and some but not all other IBV strains, was inhibited at a stage following attachment to cells but before RNA translation and transcription. The affected stage was most likely that of genome uncoating and indicates that some IBV strains enter cells by endocytosis followed by transfer to endosomes where a slightly acidic environment is required for optimum fusion of the virus and endosome membranes.

DISCUSSION

Our results show that FFWI of IBV-Beaudette was optimal at slightly acidic pH. This is in contrast to murine hepatitis virus, where the optimum pH was above 7.0 and when pH of about 6.8 was inhibitory to FFWI although not to replication[3-5]. The FFWI results obtained with IBV-Beaudette correlate with our studies using the lysosomotropic agent ammonium chloride which indicated that an acidic environment was optimal

for genome uncoating, probably within endosomes. The finding that UK/-123/82 was not inhibited by this drug and that some cells were successfully infected (as shown by fluorescence) by Beaudette even in the presence of ammonium chloride indicates that as a group IBV strains require, at most, only a mildly acidic pH for genome uncoating. In this respect they resemble some flaviviruses (Ref [6]) rather than influenza A virus (ref [7]). IBV strains unaffected by ammonium chloride would appear to have the potential for uncoating by fusion of the virus envelope with the plasma membrane. Alternatively, or in addition, uncoating may occur in endocytic vesicles but without a requirement for acidic pH. With such strains the site of uncoating probably depends on the speed of fusion with the plasma membrane relative to the rate of entry by endocytosis. When the extracellular environment is below pH7, those strains which fuse optimally at a slightly acidic pH may also be able to fuse directly with the plasma membrane.

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