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Antigenic and biochemical characterization of bovine rotavirus V1005, a new member of rotavirus serotype 10

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Bovine rotavirus (BRV) V1005 is serologically distinct from rotavirus serotypes 1, 2, 3, 4, 5, 6, 8 and 9. BRV V1005 showed cross-reactions with BRV B223, the American prototype of serotype 10 rotavirus, and with BRV E4049, a British serotype 10 isolate. BRV V1005 was, however, not neutralized by four monoclonal antibodies directed against VP7 of BRV B223. Two-way cross-reactions were observed between BRV V1005 and a reassortant rotavirus containing the VP4 from BRV UK. In addition the major tryptic cleavage product of VP4, VP5*, from BRV V1005 is indistinguishable by peptide mapping and its isoelectric point from the homologous protein of BRV UK, but is clearly different from VP5* of BRV NCDV. The peptide map of VP7 from BRV V1005 differed from that obtained for VP7 of BRV UK.

Introduction

Rotaviruses are now recognized as the most important cause of severe viral gastroenteritis in humans and young animals. Currently, 11 serotypes of rotavirus have been defined (Estes & Cohen, 1989). Six serotypes (serotypes 1, 2, 3, 4, 8 and 9) have been isolated from humans (Clark *et al.*, 1987; Matsuno *et al.*, 1985; Wyatt *et al.*, 1983). Most bovine rotaviruses (BRV) that have been typed belong to serotype 6 (Snodgrass *et al.*, 1984), but serotype 10 and occasionally serotype 8 rotaviruses have also been isolated from cattle (Snodgrass *et al.*, 1990). In addition, numerous BRV strains which are serologically distinct from serotype 6 BRV strains NCDV or UK have been described (Bellinzoni *et al.*, 1989; Bridger & Brown, 1984; Brüssow *et al.*, 1987; Ihara *et al.*, 1983; Murakami *et al.*, 1983; Ojeh *et al.*, 1984; Snodgrass *et al.*, 1984, 1990; Woode *et al.*, 1983; Zheng *et al.*, 1989). It is, however, not clear whether these new bovine strains are distinct from the established rotavirus serotypes and how they compare to each other. In the present report we give a serological and biochemical description of BRV V1005 (Bachmann & Hess, 1981), which has been characterized as a second serotype of BRV (Brüssow *et al.*, 1987).

Methods

Viruses. Growth of rotaviruses in MA-104 cell culture, radiolabelling with [^{35}S]methionine and purification of extracellular rotavirus was done as described previously (Brüssow *et al.*, 1987, 1990*a*).

Polypeptide analysis. The method of limited proteolysis analysis follows the procedure described by Cleveland *et al.* (1977). *Staphylococcus aureus* V8 protease (10 µl) (Type XVII; Sigma) at a concentration of 1 µg/µl was added to each slot. Two-dimensional PAGE was performed as described by O'Farrell (1975) with minor modifications according to Peters & Comings (1980). Gels were subjected to fluorography with Enlightning (DuPont) and exposed to X-Omat ARS X-ray films (Kodak) at −70 °C.

Hyperimmunization of guinea-pigs. Purified viral particles were emulsified in Freund's complete adjuvant (first injection), in Freund's incomplete adjuvant (second injection), or suspended in phosphate-buffered saline (third injection), and administered intramuscularly (i.m.) into guinea-pigs. Animals had been shown to be free of neutralizing antibodies to BRV UK, V1005 and simian rotavirus (SRV) SA11 (preimmune titres < 50). Sera were analysed 20 days after the last injection for neutralizing activity against 100 $TCID_{50}$ of the indicated rotavirus using the immunoperoxidase microtitre neutralization test of Gerna *et al.* (1984). Neutralizing titres are expressed as the reciprocal of the serum dilution reducing the number of infected cells by 50%.

Hyperimmunization of rabbits. Purified virus pellets containing complete virions were diluted in Tris buffer containing 2% Tween-80 to a final volume of 1 ml and emulsified in incomplete Freund's adjuvant. Each rabbit (previously shown to have a neutralizing antibody titre < 10 to UK calf rotavirus) received an i.m. injection of 1·0 ml of the emulsion at two different sites. The injections were repeated 14 days later and the rabbits were bled by cardiac puncture 7 to 10 days after the second injection. Neutralization of fluorescent focus production in MA-104 cells was used, essentially as described by Beards *et al.* (1980). Titres are expressed as the reciprocal of the serum dilution reducing fluorescent foci by 50%.

Results

Serological characterization of BRV V1005 by rabbit hyperimmune sera

BRV V1005 was not significantly neutralized by rabbit hyperimmune sera raised against established serotype 1, 3, 4, 5, 6, 7, 8 and 9 rotavirus strains (Table 1). Significant cross-neutralization of BRV V1005 was observed with rabbit hyperimmune serum to the serotype 10 prototype BRV B223 isolated in the U.S.A. (Woode *et al.*, 1983) and a recently defined British representative of serotype 10, E4046 (Snodgrass *et al.*, 1990). In addition, cross-neutralization was observed with rabbit hyperimmune serum to reassortant rotavirus DS-1 × UK, which derives VP7 from serotype 2 rotavirus DS-1 and VP4 from serotype 6 BRV UK (Greenberg *et al.*, 1983) (Table 1).

Hyperimmune serum to BRV V1005 did not neutralize serotype 1, 3, 4, 5, 7, 8 and 9 rotaviruses (Table 1); it did cross-neutralize the British serotype 10 representative, but notably did not cross-neutralize the prototype serotype 10 BRV B223 (Table 1). Rabbit hyperimmune serum to BRV V1005 showed significant cross-neutralization of BRV UK and reassortant rotavirus DS-1 × UK.

Serological characterization of BRV V1005 by guinea-pig hyperimmune sera

BRV V1005 was not significantly neutralized by guinea-pig hyperimmune sera raised against serotype 1, 2, 3, 4, 6, 8 and 10 rotavirus strains (Table 2). Hyperimmune sera to BRV V1005 did not significantly cross-neutralize serotype 1, 2 (neither strain S-2 nor DS-1), 3, 4, 5, 6, 7 and 9 rotavirus strains (Table 2). It did neutralize serotype 10 BRV B223. Of two guinea-pig hyperimmune sera to BRV V1005, one neutralized serotype 8 BRV 678 but not human serotype 8 rotavirus 69M (Table 2).

Serological characterization of BRV V1005 by monoclonal antibodies

BRV V1005 was not neutralized by monoclonal antibody (MAb) UK/7; the neutralization titre of UK/7 was >10000 both against BRV UK and BRV NCDV. BRV V1005 was not neutralized by the MAbs B223/1, B223/2, B223/4 and B223/N7, all of which neutralized BRV B223 to high titres (Table 3). Of the four MAbs to BRV B223, three also neutralized BRV E4046. In addition BRV V1005 was not neutralized by MAb 57-8 (Benfield *et al.*, 1987), which neutralized serotype 10 BRV strains B223 and E4046 (Table 3) as well as serotype 3, 4, 6 and 9 rotavirus strains (Mackow *et al.*, 1988). BRV V1005 was not neutralized (neutralization titre <10) by MAbs 2C9, IC10, 4F8 and 5B8 (Shaw *et al.*, 1985; Snodgrass *et al.*, 1990) which neutralized rotavirus serotypes 1, 2, 3 and 5 with neutralization titres of 81920, 20480, 40960 and 10240, respectively.

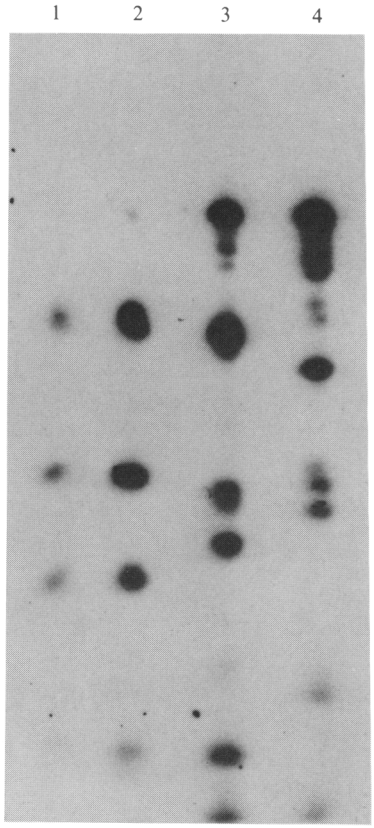

Fig. 1. Limited proteolysis analysis of the outer shell polypeptide VP5*, the major tryptic cleavage product of VP4, of rotaviruses UK (lane 1), V1005 (lane 2), NCDV (lane 3) and SA11 (lane 4) digested with *S. aureus* V8 protease.

Polypeptide analysis

The inner shell proteins VP1, VP2 and VP6 of BRV V1005 showed an identical peptide map to the corresponding polypeptides of the two BRV reference strains NCDV and UK and the SRV SA11 (Brüssow *et al.*, 1990*a*; data not shown).

We could identify two outer shell proteins in BRV V1005: VP7 and VP5*, the tryptic cleavage product of VP4 (Clark *et al.*, 1981). VP5* of BRV V1005 showed a clearly different peptide map when compared to VP5* of BRV NCDV and SRV SA11, but it was indistinguishable from the digestion pattern of VP5* from BRV UK (Fig. 1). The digestion map of VP7 from BRV V1005 differed from that obtained for VP7 from BRV UK and SRV SA11 (Fig. 2).

Table 1. *Serological characterization of BRV V1005 by rabbit hyperimmune sera*

Antiserum specificity; strain (serotype)	Neutralizing antibody titre* to indicated rotavirus strain (serotype)											
	WA (1)	DS-1 × UK (2)	RRV (3)	ST3 (4)	OSU (5)	UK (6)	Ch-2 (7)	69M (8)	WI61 (9)	B223 (10)	E4046 (10)	V1005 (?)
Wa (1)	5120†	<10	<10	<10	<10	<10	ND‡	<10	<10	<10	<10	<10
DS-1 × UK (2, VP7)	<10	640	10	40	20	<10	ND	<10	<10	<10	10	160§
RRV (3)	<10	10	20480	<10	40	40	ND	40	<10	<10	20	10
ST3 (4)	<10	<10	<10	20480	<10	<10	ND	<10	<10	<10	10	<10
OSU (5)	20	20	20	<10	20480	80	ND	640	10	<10	<10	<10
UK (6)	<10	40	<10	20	20	10240	ND	<10	40	<10	<10	10
Ch-2 (7)	ND	ND	ND	ND	ND	ND	2560	ND	ND	<10	<10	<10
69M (8)	<10	20	<10	10	2560	<10	ND	10240	10	10	<10	<10
WI61 (9)	160	<10	10	80	40	160	ND	<10	20480	160	640	160
B223 (10)	<10	<10	1280	<10	80	<10	<10	<10	80	2560	5120	640
E4046 (10)	<10	10	2560	1280	<10	<10	<10	<10	160	10240	10240	10240
V1005 (?)	<10	40	<10	<10	<10	160	<10	<10	<10	<10	40	640

* As determined in the fluorescent focus neutralization test (Beards *et al.*, 1980).

† Homologous titres are underlined.

‡ ND, Not determined.

§ Significant cross-neutralization (homologous : heterologous titre ratio <20).

Table 2. *Serological characterization of BRV V1005 by guinea-pig hyperimmune sera*

Antiserum specificity; strain (serotype)	Neutralizing antibody titre* to indicated rotavirus strain (serotype)											
	WA (1)	S-2 (2)	SA11 (3)	Hochi (4)	OSU (5)	NCDV (6)	Ch-2 (7)	678 (8)	69M (8)	WI61 (9)	B223 (10)	V1005 (?)
Wa (1)	12000†	<100	100	<100	<100	<100	<100	<100	<100	<100	<100	<50
S-2 (2)	100‡	1600	<100	100	<100	<100	<100	<100	<100	<100	<100	<50
SA11 (3)	<100	100	12000	<100	<100	<100	100	100	<100	<100	<100	<50
Hochi (4)	3200	100	200	12000	<100	<100	100	200	200	800	400	100
NCDV (6)	100	<100	200	<100	<100	12000	<100	200	100	<100	100	200
678 (8)	100	<100	<100	<100	<100	<100	<100	12000	6400	<100	<100	200
B223 (10)	<100	100	<100	<100	<100	200	100	<100	100	<100	6400	200
V1005 (?)	50	<50	<50	<50	<100	<50	100	<50	<100	<50	800	6400
V1005 (?)	200	<50	200	<50	<100	<50	100	3200	200	50	3200	6400

* As determined in the neutralization test described by Gerna *et al.* (1984).

† Homologous titre underlined.

‡ Significant cross-neutralization (homologous : heterologous titre ratio <20).

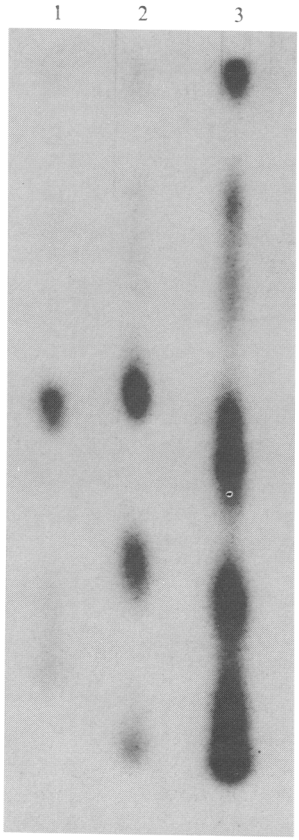

Fig. 2. Limited proteolysis analysis of the outer shell polypeptide VP7 of rotaviruses UK (lane 1), V1005 (lane 2) and SA11 (lane 3) digested with *S. aureus* V8 protease.

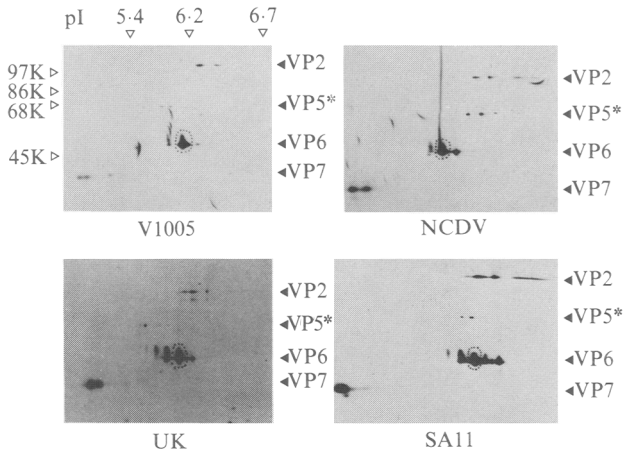

Fig. 3. Two-dimensional gel electrophoresis of [^{35}S]methionine-labelled structural polypeptides of purified double-shelled virions of rotaviruses V1005, NCDV, UK and SA11. The open arrowheads give the isoelectric point (pI) and M_rs of marker proteins. The filled arrowheads give the M_r positions of the individual rotavirus polypeptides. The major spot of VP6 polypeptide is encircled to give a reference point.

By isoelectric focusing, the VP5*s of BRV V1005 and the three reference strains were separated into as many as three spots. VP5* of BRV V1005 and BRV UK showed the most acidic pI (left of the major VP6 spot) and SA11 rotavirus had a VP5* species of intermediate pI (above the major VP6 spot), whereas BRV NCDV had the most basic VP5* species (right of the major VP6 spot) (Fig. 3). The VP7 of the BRVs separated into at least two spots with an isoelectric point at pH 4·5.

Discussion

BRV V1005 is most probably a new member of the serotype 10 rotaviruses which exhibits some antigenic drift away from the other members. It showed a two-way cross-reaction with BRV E4046 and a one-way cross-reaction with BRV B223. However, cross-neutralization of BRV V1005 with B223-specific rabbit serum was not observed with the corresponding guinea-pig serum and cross-neutralization of BRV B223 with the V1005-

Table 3. *Serological characterization of BRV V1005 by monoclonal antibodies*

Monoclonal antibody	Reference	Neutralizing antibody titre* to indicated rotavirus		
		B223	E4046	V1005
UK/7†	Snodgrass *et al.* (1990)	<10	ND‡	<10
B223/1	Snodgrass *et al.* (1990)	1280	80	<10
B223/3	Snodgrass *et al.* (1990)	163840	81920	<10
B223/4	Snodgrass *et al.* (1990)	20480	10240	<10
B223/N7	Zheng *et al.* (1989)	5120	10240	<10
57–8§	Mackow *et al.* (1988)	81920	5120	<10

* As determined in the fluorescent focus neutralization test (Beards *et al.*, 1980).
† The neutralization titre of UK/7 was >10000 against both BRV UK and BRV NCDV.
‡ ND, Not determined.
§ Monoclonal antibody raised against serotype 4 porcine rotavirus Gottfried (Benfield *et al.*, 1987) which neutralizes serotype 3, 4, 6, 9 and 10 rotaviruses *in vitro* (Mackow *et al.*, 1988; D. R. Snodgrass, unpublished results).

specific guinea-pig serum was not observed with the corresponding rabbit sera. These differences might imply more about differences in the ability to recognize certain antigens than about differences in viral antigenicity.

The absence of cross-reactivity of B223-specific MAbs with BRV V1005 indicates that the epitopes recognized by these antibodies are subject to a form of antigenic drift (Coulson *et al.*, 1985); BRV B223 and V1005 might thus represent different subtypes of serotype 10 rotaviruses. Subtypes of serotype 3 (Coulson *et al.*, 1985) and serotype 4 (Gerna *et al.*, 1988) rotaviruses have been described.

A two-way cross-reaction was also observed between BRV V1005 and rotavirus reassortant DS-1 × UK, which derives VP7 from DS-1 and VP4 from UK rotavirus (Greenberg *et al.*, 1983). In addition the rabbit, but not the guinea-pig, antiserum to BRV V1005 showed cross-neutralization of BRV UK. These serological data are not strong enough to support a serological relationship between VP4 of BRV UK and BRV V1005. It is, however, interesting to note that the tryptic cleavage product of VP4, VP5*, of rotavirus strains BRV V1005 and BRV UK were indistinguishable by peptide mapping and isoelectric focusing. On the other hand, VP5* of BRV NCDV and BRV UK, which belong to the same serotype, showed clearly different peptide maps. This biochemical difference confirms earlier observations with reassortant viruses that these two strains possess antigenically distinct VP4 antigens (Hoshino *et al.*, 1985). Thus two distinct VP4 types are associated with two BRVs that belong to the same serotype, and two similar VP4 types are associated with two BRVs that belong to different serotypes. A similar situation has recently been described for human rotaviruses. Two distinct VP4 types are associated with two human rotaviruses (KU, K8) that both belong to serotype 1 (Taniguchi *et al.*, 1989) and very similar VP4 types are associated with human rotavirus serotypes 1, 3 and 4 (Gorziglia *et al.*, 1988). The existence of two independent neutralization antigens, VP4 and VP7 (Offit & Blavat, 1986), evidently complicates the antigenic characterization of rotaviruses by the cross-neutralization test. Thus a binary system similar to that used for influenza A viruses will be more adequate for rotavirus description (Hoshino *et al.*, 1985). From work with reassortant viruses we know that the guinea-pig hyperimmune sera showed a 15-fold greater neutralizing-antibody titre to VP7 than to VP4 of the immunizing rotavirus strain (Brüssow *et al.*, 1990*b*). Development of VP4-specific monoclonal and polyclonal antibodies is therefore urgently needed to extend the current serological classification of rotaviruses.

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