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Original article

Tissue tropism of avian reoviruses is genetically determined

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Abstract – Two genome segments, M2 and S1, were preferentially selected in reassortants isolated in Vero cells. Analysis with monoclonal antibodies (MAbs) against RAM-1 strain showed that the 39-kDa protein encoded by the genome segment S1 contained epitopes involved in neutralisation of virus infectivity for both Vero and chicken kidney (CK) cells. The 39-kDa protein appeared to have two major epitopes that are attachment sites for cell receptors, one interacting only with CK cell receptors and the other with both CK and Vero cell receptors but principally Vero cell receptors. These results suggest that the strain RAM-1 may have developed an epitope for Vero cell receptors owing to mutation in the S1 genome segment, but still retained the epitope responsible for infection of CK cells. © Inra/Elsevier, Paris.

reovirus / attachment / dsRNA / σC protein

Résumé – Le tropisme des réovirus aviaires pour les tissus est déterminé génétiquement. Deux segments géonomiques, M2 et S1, ont été sélectionnés préférentiellement dans des réassortants isolés à partir de cellules Vero. L'analyse par des anticorps monoclonaux contre la souche RAM-1 a montré que la protéine de poids moléculaire 39 kDA codée par le segment géonomique S1 contenait des épitopes impliqués dans la neutralisation du pouvoir infectieux du virus à la fois pour les cellules Vero et celles de rein de poulet. Il est apparu que la protéine de poids moléculaire 39 kDa possédait deux épitopes majeurs qui sont des sites d'attachement sur les récepteurs cellulaires. L'un d'entre eux interagissait seulement avec les récepteurs des cellules de rein de poulet et l'autre avec les récepteurs de deux types de cellules mais principalement avec ceux des cellules Vero. Ces résultats suggèrent que la souche RAM-1 aurait pu développer un épitope pour les récepteurs des cellules Vero du fait d'une mutation sans le segment géonomique S1, mais qu'il est pourtant resté l'épitope responsable de l'infection des cellules de rein de poulet. © Inra/Elsevier, Paris.

réovirus / attachement / ARN double brin / protéine σC

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1. INTRODUCTION

Avian reoviruses are grouped with mammalian reoviruses in the genus *Orthoreovirus* on the basis of common physical and biochemical properties. Both groups of viruses have a genome consisting of 10-dsRNA segments. The 10-dsRNA segments migrate at different rates on polyacrylamide gels and have been classified into three size classes: large (L), medium (M) and small (S) [17, 24]. However, avian reoviruses differ from their well-studied mammalian counterparts by their lack of haemagglutinating activity [4], their ability to induce cell fusion [29], their possession of a different group-specific antigen [15, 25, 31], and their host range [19]. Avian reoviruses can be readily grown in the laboratory in primary cultures of cells of avian origin [1, 7], but they fail to replicate in most of the established mammalian cell lines [1]. Only Vero cells have been reported to be permissive hosts for the replication of certain strains of avian reoviruses [1, 3, 30]. It is probable that variation either in culture conditions [12] or between batches of Vero cell lines [8], and individual virus strain variation may be responsible for the different success rates of adaptation of avian reoviruses to Vero cells.

The aim of the work presented in this paper was to identify the dsRNA segment associated with the ability of avian reoviruses to replicate in Vero cells, by co-infection of strain RAM-1 adapted to replicate in Vero cells and strain 724 which did not [30], and comparison of the genome segments present in the reassortant and their parent viruses, with the prediction that the genome segment which allows the reassortants to replicate in the Vero cells will be preferentially selected.

2. MATERIALS AND METHODS

2.1. Viruses and cell culture

The avian reovirus strains 724 [9], Vero cell-adapted RAM-1 [30] and reassortant viruses R3 and R9 [15] were used. Avian reovirus strains 724 isolated from young chickens with tenosynovitis lesions and RAM-1 from normal Vero cells were classified into serological groups B and B/C, respectively, by Robertson and Wilcox [20]. Vero cells and primary culture of CK cells were prepared as described by Robertson and Wilcox [20].

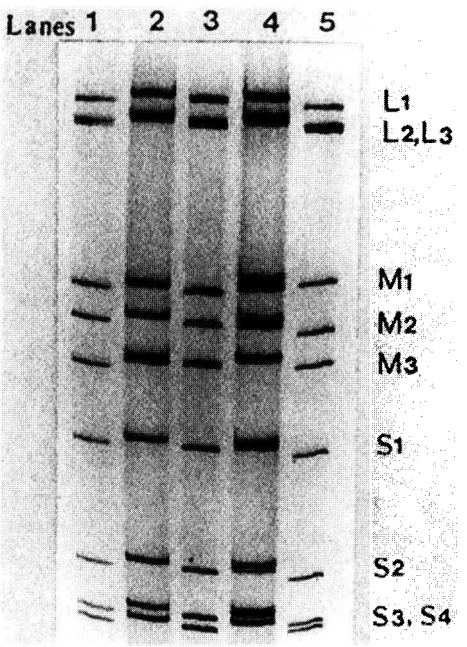

Figure 1. Genome segments of the parent virus RAM-1 and 724, and reassortant virus JR7. The genome segments were prepared and electrophoresed in a 7.5 % acrylamide gel as described in the text. Lane 1, parent virus RAM-1; lane 2, mixture of reassortant JR7 and parent virus RAM-1; lane 3, reassortant JR7; lane 4, mixture of reassortant JR7 and parent virus 724; lane 5 parent virus 724. Similar procedure was used to isolate other reassortants.

2.2. Reassortant viruses

Genetic reassortment between strains 724 and RAM-1 of avian reovirus was produced by a procedure previously described by Matsuno et al. [13]. Briefly, viruses were UV-irradiated (Sylvania germicidal lamp, 254 nm) at an incident dose rate of 34 erg/mm^2/s. Monolayers of CK were infected at a multiplicity of 10 with a 1:1 mixture of each UV-irradiated avian reovirus. After 24 h the infected cells were frozen and thawed three times, and plaque titrated in Vero cells. Isolated plaques were picked and further plaque-purified three times. The migration pattern of the dsRNA segments of the plaque-purified clones was then compared to the parent viruses using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine the parental origin of each segment (*figure 1*)

2.3. Preparation and separation of dsRNA

Monolayers of CK or Vero cells were infected with virus for 1 h at 37 °C and then incubated at 37 °C in a humidified 5 % carbon dioxide-in-air atmosphere for 24 h in Dulbecco's modified eagle's medium (DMEM) with 2 % newborn calf serum (NBCS) and 30 ng/mL actinomycin D. Cytoplasmic RNA was extracted as described by Sharpe et al. [23] and subjected to SDS-PAGE in a discontinuous Tris-glycine buffer system [10]. Samples were boiled for 2 min prior to loading onto the gels. The reovirus dsRNA samples from known and potential reassortants were loaded in the gel as follows: alone in a single lane, and mixed with an equal volume of dsRNA from the parent virus in adjacent lanes. The gels were silver stained using a commercial silver stain kit (Bio-Rad Laboratories) (*figure 1*).

2.4. Preparation of MAbs

The production of MAbs 4C5, 2C11, 2C7, 1F7, 2H4, 2G3, 6H1, 6E2 and 1G1 against RAM-1 strain of avian reovirus was previously described [31]. Preparation of polyclonal anti-RAM-1 was also described before [15].

2.5. Virus neutralisation

Neutralising activity of the MAbs was determined by a plaque-reduction neutralisation test (PRNT). One-hundred plaque-forming units (p.f.u) of virus in 0.3 mL of DMEM with 2 % NBCS was mixed with 0.3 mL dilutions of monoclonal ascites fluid or undiluted hybridoma cell culture fluid and incubated at 37 °C for 1 h. The mixture was inoculated onto Vero or CK cell monolayers in 60-mm dishes and plaqued as previously described [30]. Virus incubated with medium was used as a control. Neutralisation titres were expressed as the reciprocal of the highest dilution of antibody which reduced the plaque number by 50 % of that of the control dishes [5].

3. RESULTS

3.1. Genome segments related to adaptation of RAM-1 strain in Vero cells

Analysis of genome segments of reassortants R3, R9 and a further five reassortants designated JR2, JR7, JR9, JR24 and JR39 showed that the genome segments M2 and S1 were preferentially selected in the reassortants able to replicate in Vero cells. The overall analysis of origin of the individual genome segments in the reassortants obtained from the two parent viruses is shown in *table I*.

3.2. Neutralisation of virus infectivity

The neutralisation did not occur uniformly in Vero cells and CK cells, or against heterologous virus. The MAb 2C7 against the complex of 130- and 105-kDa proteins neutralised only the heterologous virus in

Table I. Schematic representation of reassortants derived from parent viruses RAM-1 and 724.

Migration of genome segments	Parent virus	Reassortants							Parent virus
	724	R3	R9	JR2	JR7	JR9	JR24	JR39	RAM-1
L1	_____	--------	--------	--------	--------	--------	--------	--------	*****
L2	_____	--------	--------	--------	--------	--------	--------	--------	*****
L3	_____	--------	--------	--------	--------	--------	--------	--------	*****
M1	_____	_____	_____	*****	*****	_____	*****	*****	*****
M2	_____	_____	*****	*****	*****	*****	*****	*****	*****
M3	_____	*****	_____	_____	_____	_____	*****	_____	*****
S1	_____	_____	*****	*****	*****	*****	*****	*****	*****
S2	_____	_____	*****	--------	*****	*****	_____	_____	*****
S3	_____	_____	_____	_____	*****	_____	*****	_____	*****
S4	_____	--------	--------	--------	--------	--------	--------	--------	*****
Ability to replicate in Vero cells	–	–	+	+	+	+	+	+	+

Schematic representation of the origin of individual genome segments in the reassortants obtained from the parent viruses RAM-1 and 724. (*****) genome segments of parent virus RAM-1; (_____) genome segments of parent virus 724; (--------) genome segments whose origin could not be distinguished in the reassortants.

Table II. Neutralisation of virus infectivity by monoclonal antibodies against RAM-1 virus[a].

MAb	Reactive viral protein	Molecular weight (kDa)	Location	Vero cells	CK cells	
				RAM-1	RAM-1	724
2C7	λA/λC[b]	130/105	core/surface	50[c]	<	<
1F7	λB	124	core + surface	220	<	<
2H4	λB	124	core + surface	270	<	<
2G3	λB	124	core + surface	329	<	<
4C5	μB/μBc	74/72	outer capsid	<	<	<
2C11	μB/μBc	74/72	outer capsid	<	<	<
6H1	σC	39	outer capsid	<	6 940	<
6E2	σC	39	outer capsid	230	160	<
1G1	σC	39	outer capsid	920	140	<

[a] Neutralisation determined by 50 % plaque-reduction neutralisation test.
[b] Molecular weight as determined by radioimmunoprecipitation and western blot techniques.
[c] Reciprocal of the dilution of antibody which reduced plaque number by 50 %.
< Indicates no detectable neutralisation at 1:10 dilution of antibody.

Vero cells and the neutralisation titre was 50, low in comparison to the neutralisation titre in the other MAbs which neutralised infectivity. The three MAbs against the 124-kDa protein also neutralised the homologous virus in Vero cells only. Two (6E2 and 1G1) of the three MAbs against the 39-kDa protein neutralised virus infectivity in both Vero and CK cells, but MAb 6H1 neutralised the homologous RAM-1 virus in CK cells only. The MAb against the 74/72-kDa protein was found negative for any form of neutralisation activity in either cell types (*table II*).

4. DISCUSSION

In the reassortants examined, the parental origin of the three genome segments L1, L2 and L3 could not be determined as they had a similar migration rate in both parent viruses. The role of these three genome segments in coding for a protein involved in replication of avian reovirus in Vero cells was therefore determined by the neutralisation pattern of infectivity of RAM-1 by MAbs against the 130/105- and 124-kDa proteins encoded by the genome segments L1/L3 and L2, respectively [18, 28]. The MAb against the 130/105-kDa protein did not have any neutralising activity. The three Mabs derived against the 124-kDa protein encoded by the L2 gene [18, 28] neutralised the homologous and heterologous virus in Vero cells only. The result suggests that the 124-kDa protein of avian reovirus resembled the λ2 protein of mammalian reovirus in its location and, by its neutralising activity, in function. In mammalian reovirus, antibody to λ2 neutralises infectivity by steric hindrance because of the proximity of λ2 to σ1, which is the cell attachment protein of mammalian reovirus [11]. A similar mechanism may be involved in avian reovirus because the 124-kDa protein is most likely located close to σ1, as shown by the ability of the MAbs against this 124-kDa protein to neutralise the virus in Vero cell. As MAbs to 124-kDa protein neutralised virus infectivity in Vero cells and not in CK cells, this suggests interference at the site of virus–Vero cell attachment and not at the site of virus–CK cell attachment. Furthermore, the genome segments L1, L2 and L3 are considered to be responsible for encoding for the most highly conserved viral proteins [6], they are probably unlikely to be responsible for the differing ability of avian reoviruses to replicate in Vero cells. In mammalian reoviruses these genome segments are responsible for encoding large proteins mainly found within the capsid [2, 14].

Amongst the six reassortants able to replicate in Vero cells, it was found that the two genome segments, M2 and S1 always originated from the parent virus RAM-1 able to replicate in Vero cells and never from the parent virus unable to replicate in Vero cells. In mammalian reoviruses, the S1 genome segment encodes the σ1 surface protein which is involved in attachment to cells [11]. The S1 genome segment of avian reovirus codes for the 39-kDa σC protein [18, 21, 28] which is present in a minute quantity in the outer capsid [22] and is responsible for type-specificity [15], fusion of the transfected cells [27] and infected cells [16] suggesting a role analogous to the cell attachment protein σ1 of mammalian reovirus. We therefore assume that the S1 genome segment of avian reovirus was also responsible for coding an attachment protein homologous for the Vero cell receptors, and that this enabled the infection and replication of virus in Vero cells.

This probability was analysed with the four MAbs against parent virus RAM-1. The neutralisation pattern of infectivity of RAM-1 by MAbs showed that of the 74/72- and 39-kDa proteins encoded by the genome segments M2 and S1, respectively [18, 28], only the 39-kDa protein was involved in neutralisation of virus infectivity in both Vero and CK cells.

The three MAbs against the 39-kDa protein exhibited a different ability to neutralise the infectivity of the homologous RAM-1 virus in Vero and CK cells. The MAb 6H1 neutralised the virus to a higher titre than the other two MAbs in CK cells, suggesting that this MAb reacted with an epitope which is directly involved in the infectivity of the virus for these cells, but it did not neutralise virus infectivity in Vero cells. In contrast, MAb 6E2 and 1G1 neutralised the infectivity of RAM-1 for both CK and Vero cells. The neutralisation titre of MAb 6E2 was approximately the same in CK and Vero cells, and the neutralisation titre of MAb 1G1 was approximately six-fold greater in Vero cells than in CK cells.

In conclusion, based on these observations, the 39-kDa protein encoded by the S1 genome segment appeared to have two major epitopes that are attachment sites to cell receptors, one recognised by 6H1 which interacts only with CK cell receptors and the other located close to the epitope recognised by 1G1 which interacts with both CK and Vero cell receptors, but principally Vero cell receptors. As the MAb 6E2 is of IgM isotype and as it is known that IgM can have a greater steric effect because of its oligomeric nature [26], it is possible that it recognised an epitope located between the epitopes recognised by 1G1 and 6H1 and neutralised virus by steric hindrance rather than by direct involvement with the major neutralising epitopes.

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