

THE IMMUNOLOGICAL RESPONSE TO INTACT AND DISSOCIATED BLUETONGUE VIRUS IN MICE

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ABSTRACT

OELLERMANN, R. A. & CARTER, P., 1977. The immunological response to intact and dissociated bluetongue virus in mice. *Onderstepoort Journal of Veterinary Research*, 44 (4), 201-204 (1977).

Antigenic fractions of bluetongue virus were separated by ultracentrifugation in Tris-buffered CsCl gradients at pH 6, 7 or 8 and the bluetongue virus polypeptide composition of the bands isolated from these gradients was monitored by polyacrylamide gel slab electrophoresis.

The immunological response to these fractions in mice was determined by a haemolytic plaque-forming cell assay, using sheep erythrocytes onto which intact bluetongue virus was adsorbed as lytic indicator cells. Isolated outer layer bluetongue virus polypeptide 2, from gradients at pH 6, and polypeptides 2 and 5, from gradients at pH 7, produced a strong primary IgM plaque-forming cell response. The subviral particles of density 1,39 g.cm⁻³ and the bluetongue virus core particles of density 1,42 g.cm⁻³ also stimulated an IgM response at least as strong as that to intact bluetongue virus of density 1,38 g.cm⁻³. The isolated bluetongue virus fractions therefore appear to maintain their immunogenic integrity as effectively as those of intact bluetongue virus. The pattern of the immune response to bluetongue virus type 4 is similar to that of type 10.

Résumé

RÉACTIONS IMMUNITAIRES DE LA SOURIS AUX VIRUS INTACT ET DISSOCIÉ DE LA FIÈVRE CATARRHALE DU MOUTON

Des fractions antigéniques du virus de la fièvre catarrhale du mouton (FCM) ont été séparées par ultra-centrifugation dans des gradients de CsCl Tris-tamponnés aux pH 6, 7 ou 8 et la composition polypeptidique du virus FCM dans les bandes isolées à partir de ces gradients a été suivie par électrophorèse sur plaques de gel de polyacrylamide.

Les réactions immunitaires à ces fractions chez la souris ont été déterminées par un test hémolytique à cellules formatrices de plaques, utilisant comme cellules indicatrices de lyse des érythrocytes de mouton adsorbant du virus FCM intact. Le polypeptide 2 de la couche externe du virus FCM, isolé de gradients au pH 6, et les polypeptides 2 et 5, isolés de gradients à pH 7, ont élicité une forte réaction primaire IgM des cellules formatrices de plaques. Les particules subvirales de densité 1,39 g.cm⁻³ et les particules du noyau du virus FCM de densité 1,42 g.cm⁻³ ont également provoqué une réaction IgM au moins aussi forte que celle provoquée par le virus FCM intact, de densité 1,38 g.cm⁻³. Il semble donc que les fractions du virus FCM maintiennent après séparation leur intégrité immunogénique aussi efficacement que les fractions du virus intact. Le schéma de la réaction immunitaire au virus FCM type 4 est semblable à celui qui correspond au type 10.

INTRODUCTION

Bluetongue virus (BTV) has been shown to consist of 7 polypeptides, 2 of which are present as a diffuse protein layer surrounding the capsid (Verwoerd, Els, De Villiers & Huismans, 1972). Recently a haemolytic plaque technique for the detection of BTV specific antibody-forming cells was described (Oellermann, Carter & Marx, 1976a), who also used this technique in a study of the kinetics of the IgM and IgG response to BTV in mice. To extend these studies to BTV fractions isolated from purified virus preparations, they investigated the immune response to intact and dissociated BTV.

MATERIALS AND METHODS

Antigen preparation

Virulent strains of BTV Types 4 and 10 were used in this investigation. The production, purification on sucrose gradients and selective dissociation of BTV in CsCl was carried out as described by Verwoerd *et al.* (1972). Isopycnic density gradient ultracentrifugation of the sucrose-purified BTV in 0,2M Tris-buffered CsCl provided the following antigenic fractions:

- Intact BTV of density 1,38 g.cm⁻³ at pH 8;
- BTV core particles of density 1,42 g.cm⁻³ and outer layer polypeptides 2 and 5 of density 1,29-1,32 g.cm⁻³ at pH 7;
- Subviral particles of density 1,39 g.cm⁻³ and outer layer polypeptide 2 of density 1,29-1,32 g.cm⁻³ at pH 6.

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Received 7 June 1977—Editor

To locate the BTV fractions on the CsCl gradients after centrifugation, 3-drop fractions were collected from the bottom of the centrifuge tube. After the density of CsCl in the different fractions had been determined, 1,2 ml of 0,002M Tris-HCl at pH 8,8 was added to each and the adsorbance at 260 nm measured.

Assay of the immunological response

Groups of 3 ten-week-old male albino mice from the colony maintained at the Institute were immunized intraperitoneally with the BTV antigenic fractions at a concentration of approximately 40 µg protein per mouse. After 4 days the primary IgM response was determined by the BTV haemolytic plaque-forming cell (PFC) assay as previously described (Oellermann *et al.*, 1976a, b).

For each experiment plaque counts were made at each of 3 serial 3-fold spleen cell dilutions. The results presented are the mean ± standard error (SE) PFC per 10⁷ spleen cells calculated from 3 or more experiments.

Polyacrylamide gel electrophoresis

Electrophoretic separation of BTV polypeptides was carried out on a 5 mm thick gel slab as described previously (De Villiers, 1974; Verwoerd *et al.*, 1972). However, electrophoresis was carried out at 30V, a capacitance of 0,5 mfd, and a rate of 1 000 pulses per sec for 22 h.

RESULTS

Isopycnic sedimentation pattern of fractionated BTV CsCl gradients

Antigenic fractions of BTV were isolated from CsCl density gradients at pH 6, 7 and 8 after ultracentri-

fugation. Typical sedimentation patterns of BTV 10V at 260 nm are illustrated in Fig. 1.

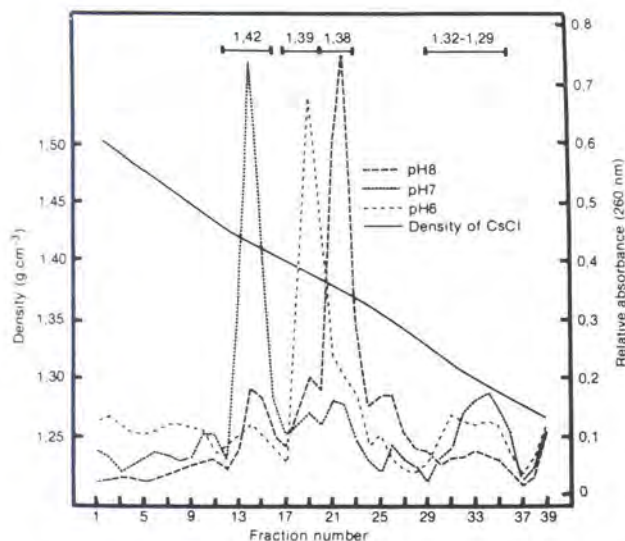


FIG. 1 Isopycnic sedimentation patterns of BTV 10V after ultracentrifugation in CsCl gradients at pH 8, pH 7 and pH 6

The major band at pH 8 representing intact BTV had a density of 1.38 g.cm^{-3} . At pH 7, core particles of density 1.42 g.cm^{-3} and at pH 6 subviral particles of density 1.39 g.cm^{-3} were the major components respectively. At all 3 pH values, dissociated viral proteins were isolated from a broad band having a density of $1.32-1.29 \text{ g.cm}^{-3}$. In all cases a number of other minor bands were also found. The comparative adsorbance curves of BTV 4V gave basically the same sedimentation patterns as shown in Fig. 1.

Polypeptide composition of CsCl-isolated BTV fractions

The polypeptide composition of the different BTV fractions isolated after CsCl ultracentrifugation was determined by polyacrylamide gel slab electrophoresis.

Intact virus represented by density band 1.38 at pH 8 was composed of 7 polypeptides. At pH 7, the core particles banding at 1.42 showed that polypeptides 2 and 5 were removed and could be recovered in the protein band of 1.29-1.32. At pH 6, the 1.39 band of subviral particles showed polypeptide 2 to be absent with its concomitant recovery in the protein band 1.29-1.32. This protein band was slightly contaminated with polypeptide 5 as a result of partial dissociation of some BTV to core particles, as is evident from Fig. 1. These results confirm earlier findings by Verwoerd *et al.* (1972).

In vivo immunological responses to CsCl-isolated BTV fractions

The primary IgM PFC responses to the different BTV antigenic fractions were determined 4 days after immunization of the mice. The results are shown in Table 1.

Although differing in magnitude, all the antigenic fractions used elicited strong primary immune responses in mice, the pattern of the immune response of BTV 4V being similar to that of BTV 10V. The most significant observation is that isolated outer-layer BTV proteins retain their antigenicity.

TABLE 1 The IgM PFC responses to different antigenic fractions of BTV types 4 and 10 isolated from CsCl density gradients

BTV adsorbed to SRBC	Antigenic fraction	pH of CsCl gradient	IgM PFC (\pm SE) per 10^7 spleen cells
BTV 10,	BTV 10 1,38	8	$1\ 422 \pm 301$
	BTV 10 1,39	6	$1\ 601 \pm 275$
	BTV 10 1,42	7	$2\ 357 \pm 389$
	BTV 10 1,29-1,32	6	725 ± 186
	BTV 10 1,29-1,32	7	923 ± 203
BTV 4,	BTV 4 1,38	8	$1\ 628 \pm 298$
	BTV 4 1,39	6	$1\ 793 \pm 312$
	BTV 4 1,42	7	$2\ 149 \pm 343$
	BTV 4 1,29-1,32	6	950 ± 196
	BTV 4 1,29-1,32	7	$1\ 140 \pm 248$

DISCUSSION

To detect BTV specific antibody-forming cells by the PFC assay, sheep erythrocytes onto which intact BTV has been adsorbed act as the lytic indicator cells. In the present investigation, therefore, the immune response to the BTV fractions of different densities is a measure of the response to immunogenic determinants that are available in the intact virus. The results clearly show that all the BTV antigenic fractions tested elicited a strong immune response as determined by the PFC assay. These BTV fractions, therefore, appear to maintain their conformation and essential immunogenic determinants as they exist on the intact BTV. Dissociation of BTV by density-gradient ultracentrifugation in Tris-buffered CsCl is therefore a useful technique in a study of the contribution by the different viral polypeptides to the BTV immunological response.

During the course of this investigation, it was found that some variation in the CsCl dissociation patterns at pH 6, 7 and 8 occurred. Although the major bands at the respective pH values were always obtained, the relative intensities of the minor bands showed a marked variation, more frequently so with BTV 10V than with BTV 4V. Similar observations were made on BTV by earlier workers (Verwoerd, personal communication), but no explanation can be offered for this phenomenon.

The protein band isolated from CsCl gradients at pH 6 consisted mainly of polypeptide 2. Although there was a small amount of contamination by polypeptide 5, its contribution to the immune response *in vivo* is considered to be insignificant as the total antigen concentration of the fraction used for immunization was $40 \mu\text{g}$ per mouse.

During preliminary experiments (unpublished observations, 1975) in the development of the PFC assay (Oellermann *et al.*, 1976a), it was found that not only intact BTV of density 1.38 g.cm^{-3} adsorb onto sheep erythrocytes but also subviral BTV particles of density 1.39 g.cm^{-3} , but not core particles of density 1.42 g.cm^{-3} . Polyacrylamide gel electrophoretic separation showed that polypeptide 2 was removed from the subviral particles and polypeptides 2 and 5 from the core particles. Polypeptide 5 therefore seems to play a significant role in the adsorption of BTV to cells. Although this adsorption is speculatively considered to be non-specific, it could indicate which

polypeptide is involved in the specific adsorption of BTV to the receptors on immunocompetent lymphocytes.

The most significant contribution emerging from this study is the demonstration for the first time that dissociated outer layer BTV polypeptides elicit the synthesis of antibodies which can adsorb to intact BTV on sheep erythrocytes resulting in haemolytic PFC formation. The immunogenic integrity of these polypeptides is therefore essentially maintained after removal from the virion. This, together with the current indications that polypeptide 2 is probably responsible for BTV serotype specific neutralizing antibodies (Huisman & Howell, 1973; De Villiers, 1974), indicates the need to investigate the possibility of vaccination by purified or synthetic BTV polypeptides.

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