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KINETICS OF THE IgM AND IgG IMMUNOLOGICAL RESPONSE TO SHEEP ERYTHROCYTES AND BLUETONGUE VIRUS IN MICE

R. A. OELLERMANN, P. CARTER and M. J. MARX, Veterinary Research Institute, Onderstepoort

ABSTRACT

OELLERMANN, R. A., CARTER, P. & MARX, M. J., 1976. Kinetics of the IgM and IgG immunological response to sheep erythrocytes and bluetongue virus in mice. *Onderstepoort Journal of Veterinary Research* 43 (4), 185–188 (1976).

The IgM and IgG response of mice to sheep erythrocytes (SRBC) and bluetongue virus (BTV) was determined by means of haemolytic plaque assays. Maximum primary IgM response to SRBC occurred after 4 days but declined rapidly to 4% of the maximum by Day 9. A lag period of about 2 days was observed in the appearance of IgG haemolytic plaque-forming cells (PFC) but they reached a maximum after 6–9 days. Secondary immunization resulted in the stimulation particularly of IgG PFC and from Day 6 onwards IgG predominated in the immunological response.

The IgM response to BTV was remarkably similar to that observed when SRBC were used as antigen. IgG PFC, however, appeared within a day of the IgM, reaching a peak on Days 4–5. From then onwards, IgG PFC predominated in the response. At BTV concentrations of up to 10 μg per mouse, the virulent strain of BTV type 3 produced the weakest response. At higher antigen concentrations there was very little difference in the response to the serotypes tested, although the virulent strain of BTV type 4 tended to produce the strongest response.

Résumé

LA RÉPONSE IMMUNITAIRE CINÉTIQUE EN IgM ET IgG CHEZ LA SOURIS AUX GLOBULES ROUGES DU MOUTON ET AU VIRUS DE LA PESTE OVINE

En employant des essais en plaques haemolytiques, les auteurs ont mis au jour la réponse immunitaire en IgM et IgG aux globules rouges du mouton (GRM) et au virus de la peste ovine (VPO). La réponse en IgM aux GRM est au maximum après 4 jours en diminuante rapidement à 4 p.100 du maximum au 9em jour. Une période de retard de 2 jours environ s'est manifestée dans l'apparition de cellules formatrices de plaques haemolytiques (CFPH) aux IgG, ces cellules atteignantes un maximum après 6–9 jours. L'immunisation sécondaire a surtout déterminée une excitation des CFPH aux IgG et la prédominance des IgG aprés le 6 em jour dans la résponse immunitaire.

La réponse immunitaire en IgM au VPO ressemble fortement celle déterminée par les GRM. Les CFPH aux IgG ont pu être détectées un jour après les IgM, en atteignantes un maximum à 4–5 jours. Dorénavant, les CFPH aux IgG ont dominé la réponse immunitaire. La souche virulente de type 3 du VPO aux concentrations atteignantes 10 μg par souris, a suscité la réponse la plus faible. Des concentrations antigéniques plus élevées des différentes sérotypes ont suscité des réponses peu différentes, mais la souche virulente de type 4 du VPO tend à susciter la réponse la plus forte.

INTRODUCTION

A technique for the detection and enumeration of haemolytic antibody plaque-forming cells (PFC) was developed by Jerne, Nordin & Henry (1963). This method permits the detection in gels of single cells actively releasing haemolysins which, in the presence of complement, cause distinct areas of lysis termed plaques. The direct technique as described by Jerne *et al.* (1963) is limited to the detection of 19S IgM, i.e. high-efficiency haemolytic antibody PFC, whereas 7S-non-IgM low efficiency haemolytic antibody PFC may be identified through an indirect plaque assay (Dresser & Wortis, 1965; Sterzl & Riha, 1965; Wortis, Taylor & Dresser, 1966, 1968; Plotz, Talal & Asofsky, 1968; Sell, Park & Nordin, 1970; Pierce, Johnson, Gershon & Asofsky, 1971). To detect indirect IgG PFC, a heterologous anti-IgG serum is required to render the IgG produced haemolytic, thereby giving rise to a visible plaque on the addition of complement.

These techniques have greatly advanced the study of the kinetics of the appearance of different antibody synthesizing cells. The mouse-heterologous erythrocyte system has played a central role in the elucidation of the IgM and IgG immune response following single or multiple immunizations (Jerne *et al.*, 1963; Plotz *et al.*, 1968; Wortis, Dresser & Anderson, 1969; Sell *et al.*, 1970). It has also been shown that, depending on the antigen, quantitative and qualitative differences exist in the immune response between different strains of mice (Warner, Vaz & Ovary, 1968).

The haemolytic plaque assay was recently modified for the detection of BTV-specific antibody secreting cells (Oellermann, Carter & Marx, 1976). Very little is known of the basic immunological response to BTV of which 16 serotypes are known (Howell, Kümm & Botha, 1970). In this study the IgM and IgG response to single and multiple injections of BTV in mice was investigated. No comparative data on the mouse-SRBC system in animals from the local colony are available but this aspect was also investigated.

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MATERIALS AND METHODS

Antigen preparation

Suspensions of SRBC were prepared by the method previously described by Oellermann (1974a).

Virulent (V) strains of BTV types 3, 4 and 10 and the egg-attenuated (A) strain of BTV 10 were used in this investigation. The production and purification of live BTV was carried out by the method of Verwoerd, Els, De Villiers & Huismans (1972). The concentration of purified BTV suspensions was determined spectrophotometrically by the method of differential absorbance (Oellermann, 1974b).

Assay of the immunological response

Ten-week-old male albino mice from the local colony were used throughout. They were immunized intraperitoneally with 5×10^8 SRBC per mouse and the immunological response was assayed as previously described (Oellermann, 1974a).

Similarly, mice were immunized with 30 μg BTV except in the study of the dose response where 0–100 μg BTV was injected per mouse. The immunological response was determined by the BTV specific PFC assay described in detail by Oellermann *et al.* (1976). IgM PFC were scored directly after incubation in in the presence of a 1:15 dilution of complement.

The difference in numbers of PFC obtained in the presence or absence of a 1:2500 dilution of goat anti-mouse IgG serum* was taken to represent IgG PFC.

For each experiment, plaque counts were made in duplicate at each of 3 serial 3-fold spleen cell dilutions. The results presented are the mean PFC per 10^7 spleen cells calculated from triplicate experiments.

Results

Immunological response to SRBC

No experimental data on the immunological response of mice from the local colony to SRBC are available for comparison with results reported in the literature. The primary and secondary IgM and IgG response to SRBC was therefore determined over a period of 16 days after the primary immunization. The results obtained are given in Fig. 1.

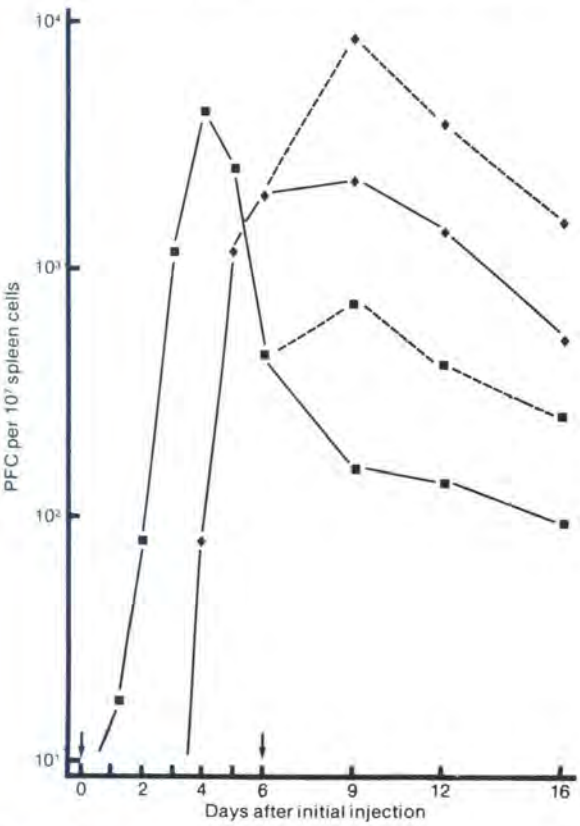

FIG. 1 The ———— primary and - - - - - - - secondary ■ IgM and ◆ IgG response to SRBC in mice. Arrows at Days 0 and 6 indicate primary and secondary immunizations, respectively

After the initial immunization, the maximum IgM response was observed after 4 days. This was followed by a rapid decline in the numbers of PFC up to Day 9. Thereafter, only a slight decrease was observed. IgG PFC began to appear on Day 4 and increased rapidly to Day 6. A slight increase followed up to Day 9, whereupon the numbers of IgG PFC dropped until the experiment was terminated on Day 16.

Upon the second immunization on Day 6, IgM PFC increased slightly to Day 9, thereafter decreasing to Day 16. However, a marked increase in the IgG PFC to Day 9 was followed by a decrease up to Day 16.

* Miles Laboratories (Pty) Ltd, Cape Town

TABLE I The contribution of IgM and IgG PFC to the immunological response to SRBC in mice

Day after initial immunization	Primary (P) or secondary (S) response	IgM as % of IgM response on Day 4	IgG as % of	
			a. IgG response on Day 6	b. IgM + IgG
4	P	100	—	2
6	P	10	100	70
9	P	4	120	94
	S	17	350	92
16	P	3	22	85
	S	8	90	53

To determine the contributions of the IgM and IgG classes of immunoglobulins to the immunological response against SRBC in mice, the data presented in Table 1 were compiled from the time response curves in Fig. 1. From these data it is evident that the IgM response is of relatively short duration. On Day 16 the primary IgM response represents only 3% and for the secondary reaction 8% of the 4-day IgM peak response. On the same day, however, the IgG response was more pronounced, representing 22% for the primary reaction and 90% for the secondary reaction of the 6-day IgG response. From Day 6 onwards, IgG predominated in the immunological response, representing 85% for the primary reaction and 53% for the secondary reaction of the respective total (IgM+IgG) response on Day 16.

IgM and IgG time response to BTV

During the development of the BTV PFC assay (Oellermann, *et al.*, 1976), mice, used as the experimental animals, demonstrated their usefulness in a fundamental study of the immunological response to BTV. As no basic information on the immunological response to BTV in mice was available, it was necessary to determine the IgM and IgG response. Primary, secondary and tertiary immunizations were administered on days 0, 10 and 17, respectively, and the responses were determined daily over a period of 21 days after the primary injection. The results are shown in Fig. 2.

As with the response to SRBC, the rapid increase to a peak in IgM PFC by Day 4 was followed by a marked decline until Day 8. Thereafter, no marked changes were observed except that on Days 11 and 12 increased IgM PFC were detected. Unlike the response to SRBC, IgG PFC appeared much earlier, reaching a peak on Days 4–5 and declining to Day 8. During Days 9–12, an increase in response was observed, whereafter it declined again to Day 21. From Day 5 onwards IgG PFC predominated in the response.

Secondary and tertiary immunizations resulted in a stimulation of both the IgM and IgG PFC to values greater than those observed during the primary and secondary reactions, respectively.

To determine the relative contributions of IgM and IgG to the immunological response against BTV, the data in Table 2 were compiled from Fig. 2.

A comparison of the data in Tables 1 and 2 shows a remarkable similarity between the IgM response to both SRBC and BTV. The relative contribution by IgG to the total (IgM+IgG) response was fairly constant during the experimental period of 8–20 days when BTV was used as antigen. With SRBC as antigen, however, there appeared to be a maximum contribution by IgG around Day 9.

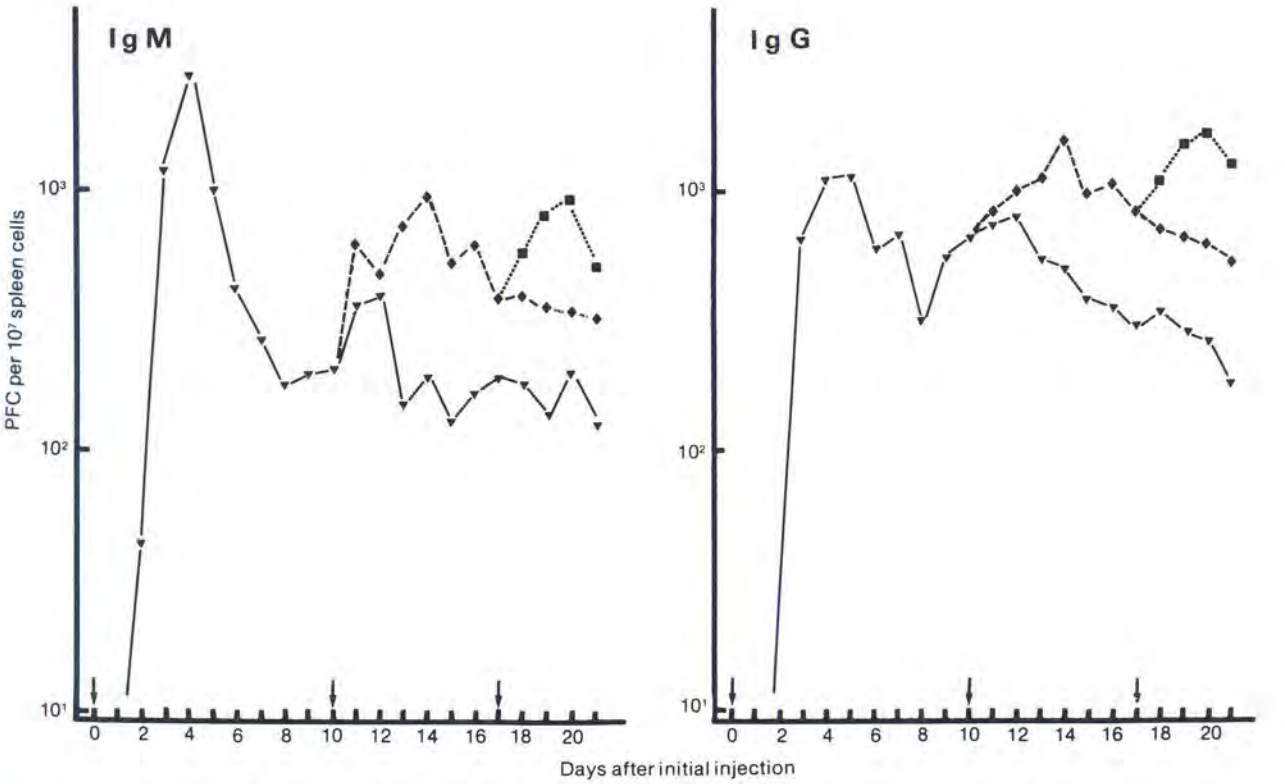

FIG. 2 The ▼———▼ primary, ◆- - - - - - -◆ secondary and ■·····■ tertiary IgM and IgG response to BTV in mice. Arrows at Days 0, 10 and 17 indicate primary, secondary and tertiary immunizations, respectively

TABLE 2 The contribution of IgM and IgG PFC to the immunological response to BTV 10A in mice

Days after initial immunization	Primary (P), secondary (S) or tertiary (T) response	IgM as % of IgM response on Day 4	IgG as % of	
			a. IgG response on Day 5	b. IgM + IgG
4	P	100	99,5	28
8	P	7	29	64
12	P	15	72	66
	S	17	89	68
16	P	6	32	67
	S	23	100	63
20	P	7	23	55
	S	12	56	65
	T	32	146	64

Dose response to different serotypes of BTV

Type 10A was used in the foregoing experiments on BTV but, to extend the knowledge on the immunological response against BTV, dose response curves for BTV serotypes 3V, 4V, 10V and as well as 10A were determined. The results are presented in Fig. 3.

With increasing antigen concentrations of up to 10 μg per mouse, the PFC response to the different serotypes of BTV showed a marked increase. At these low antigen concentrations the response to serotype 3V was weaker than that to the others. With antigen concentrations above 10 μg per mouse, no real differences between the serotypes could be detected, although type 4V tended to give the highest response.

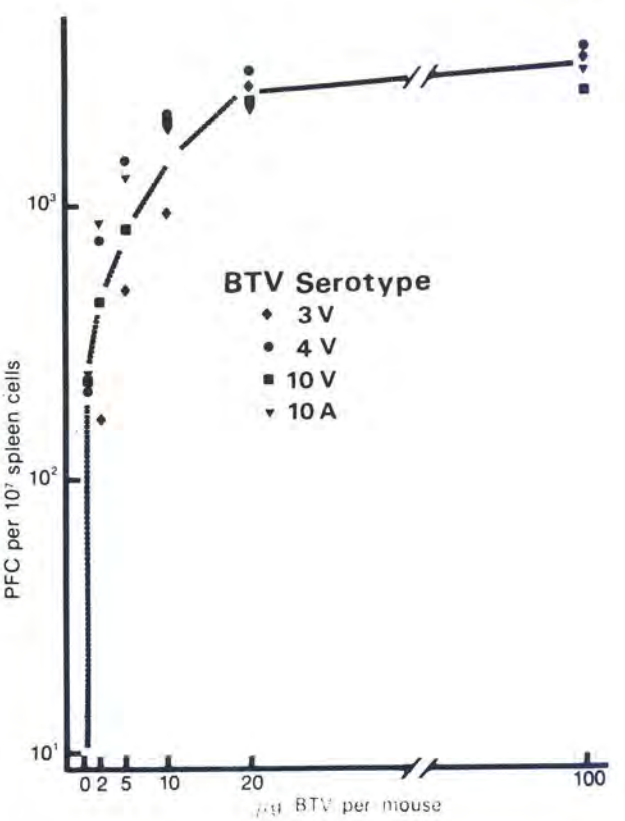

FIG. 3 IgM PFC response to varying concentrations of different serotypes of BTV in mice

Discussion

It was found that the primary immunization with SRBC resulted in the rapid appearance of IgM PFC followed 2 days later by the appearance of IgG PFC. IgM PFC reached a peak after 4 days and, although the response was of relatively short duration, it did not disappear completely over the experimental period of 16 days. From Day 6 onwards, IgG PFC response was the major contributor to the immunological response in both the primary and secondary reactions. Compared to that of IgM PFC, the IgG PFC response was more sustained, reaching a maximum contribution at approximately Day 9. Thus, in general, the immunological time response curves to SRBC in mice determined in this investigation agree well with those reported by Jerne *et al.* (1963), Plotz *et al.* (1968), Wortis *et al.* (1969) and Sell *et al.* (1970) in spite of possible differences which may exist between different strains of mice (Warner *et al.*, 1968).

In this study, the first to report on the IgM and IgG immunological response to BTV, the mouse was found to be a suitable experimental animal but the data obtained would have to be compared with those on sheep which are the conventional experimental animals for studies on BTV.

The kinetics of the IgM PFC response against BTV in mice are remarkably similar to those reported using SRBC as antigen. According to Warner *et al.* (1968), complement is fixed most efficiently by IgM and, to a lesser extent, by IgG 2a and 2b antibody. The fact that the complement fixing antibody response to BTV in sheep is of relatively short duration (Howell, personal communication, 1976), indicates the possible involvement of IgM, but whether this is paralleled in the mouse must, of course, be verified in a more detailed study.

IgG PFC appear within a day of the IgM PFC. Since this is different from the response to SRBC, where a lag of 2 days was observed, it would be necessary in a future study to resolve the subclasses of IgG PFC and determine whether a correlation exists between the PFC response and serum neutralizing antibody titres against BTV.

A comparison of the IgM PFC response to different serotypes of BTV showed that type 3V gave the weakest response at concentrations up to 10 μg antigen per mouse, but, at antigen concentrations of 20–100 μg per mouse, there was little difference in response to the 4 serotypes, except that the PFC response to BTV 4V tended to be slightly higher than the others. From earlier studies on neutralizing antibodies in sera of sheep (Hübschle, personal communication, 1976), type 3A apparently produced the weakest response. Types 4A and 10A appeared to elicit the strongest response with very little difference in neutralizing antibody titres between these 2 serotypes. These data tend to be supported by the results from the present investigation in spite of the different criteria employed to assay the immunological response.

When the intraperitoneal route of immunization in mice is used, as in this investigation, live BTV does not multiply *in vivo*. The mouse-BTV system therefore offers a number of advantages over the sheep-BTV system for a detailed study of the immunological response to the different serotypes of BTV. The fact that the sheep acts as natural host to BTV would require that results derived from any future comparative studies between the 2 viral host systems be carefully scrutinized.

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