The Influence of Complement on the Neutralization of Infectious Bovine Rhinotracheitis Virus by Globulins Derived from Early and Late Bovine Antisera

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ABSTRACT

Calves were inoculated at four week intervals with infectious bovine rhinotracheitis virus (IBRV). Sera were obtained at eight days (early sample) and 21 days (late sample) after each inoculation. Kinetic neutralization was carried out with 7S and 19S globulins derived from these sera, both in the presence and in the absence of guinea pig complement (C). In all instances the 19S neutralizing antibody (AB) was dependent on C for neutralization of IBVR. However, C dependency was not observed with any of the 7S preparations. Neutralizing activity was readily detected in 19S globulins from the early sera after primary and subsequent inoculations but not in any of the late sera. Early sera collected after primary inoculation did not contain any 7S neutralizing AB but it was present in all the other sera tested. The 7S AB when present was always at a considerably higher concentration than 19S AB. Thus it may be possible to determine whether cattle have recently been exposed to IBRV when paired serum samples are not available by determining the presence of C-dependent 19S globulins. In addition, by comparing 19S and 7S levels, a distinction may be made between primary and secondary responses to IBRV.

RÉSUMÉ

L'auteur a inoculé des veaux, à deux reprises et à quatre semaines d'intervalle, avec le virus de la rhino-trachéite infectieuse bovine. Il préleva des échantillons de sérum, le huitième et le 21e jour après chacune des inoculations. Il effectua ensuite une épreuve de neutralisation cinétique avec les globulines 7S et 19S de ces échantillons, tant en présence qu'en l'absence de complément de cobaye. Dans tous les cas, l'anticorps neutralisant 19S s'avéra dépendant du complément de cobaye pour neutraliser le virus, contrairement à ce qui se produisit pour l'anticorps 7S. L'activité neutralisante des globulines 19S se manifesta dans les échantillons de sérum prélevés le huitième jour après les deux inoculations, mais non dans ceux du 21e jour. Aucun des échantillons prélevés le huitième jour après la première inoculation ne recelait de globulines 7S, contrairement à tous les échantillons prélevés ultérieurement. Lorsque présentes, les globulines 7S l'étaient toujours dans une proportion beaucoup plus considérable que les globulines 19S. Par conséquent, il pourrait s'avérer possible de déceler un cas récent de rhino-trachéite infectieuse bovine, même en l'absence d'une paire d'échantillons de sérum, pourvu qu'on se borne à rechercher la présence des globulines 19S. De plus, une comparaison de la teneur du sérum en globulines 19S et 7S pourrait permettre de différencier entre une première et une deuxième réaction à l'endroit du virus de la rhino-trachéite infectieuse bovine.

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INTRODUCTION

Infectious bovine rhinotracheitis virus (IBRV) is associated with respiratory disease (11), genital disease (6), encephalitis (2), conjunctivitis (1) and abortion (9) in cattle. Diagnosis of disease due to IBRV is best achieved by virus isolation from animals with typical disease (16). However, virus isolation is an expensive and often inefficient and impractical method of diagnosis. Diagnosis can also be made by demonstrating an increase in antibody titer in affected animals when acute stage serum and serum from the convalescent phase are compared (16). The latter method also has several disadvantages. Paired serum samples are frequently not available or, at best, their collection can be time consuming and inconvenient. In a previous study it was shown that bovine sera collected from cattle soon after primary exposure to IBRV contained complement (C)-dependent antibody but that late serum samples and even early sera after a secondary exposure to the virus contained little, if any, C-dependent antibody (13). However, whole sera were employed in the latter study which makes it difficult to draw definitive conclusions since non-complement dependent neutralizing antibodies frequently mask the presence of the complement dependent antibodies (12). Thus, in the absence of information of what particular globulins are involved in the neutralization of IBRV in bovine sera, the use of serology for the diagnosis of IBR becomes confusing. It has been reported that the neutralization of IBRV by 19S and early 7S derived from rabbit anti-IBRV sera was C-dependent while late 7S antibody was only slightly enhanced by C (12). It was the purpose of the work reported here to characterize the immune response of cattle to IBRV more fully and to determine whether this information could be useful in the diagnosis of IBRV infections.

TABLE I. Neutralization Rate Constants (K) of Globulins Derived from Early Bovine Sera after Primary Exposure to IBRV

Calf	Globulin	K Without C[a]	K With C	K Ratio[b]
37	19S	0.07	0.79	12.0
49	19S	0.09	0.67	7.2
37	7S	0.16	0.07	0.5
49	7S	0.09	0.09	1.0

[a]Guinea pig complement
[b]Ratio of K value with C to K value without C

TABLE II. Neutralization Rate Constants (K) of Globulins Derived from Late Bovine Sera after Primary Exposure to IBRV

Calf	Globulin	K Without C[a]	K With C	K Ratio[b]
37	19S	0	0.11	
49	19S	0	0.06	
40	19S	0.02	0.11	6.0
37	7S	1.25	1.36	1.1
49	7S	1.16	1.18	1.0

[a]Guinea pig complement
[b]Ratio of K value with C to K value without C

MATERIALS AND METHODS

Medium and Cells

Madin Darby bovine kidney (MDBK) line cells were used in this experiment. The cells were propagated in Eagle minimum essential medium with Earle salts, L-glutamine and nonessential amino acids (MEM) supplemented with fetal calf serum (FCS) as described previously (12).

Virus and Virus Assay

A vaccine strain of IBRV was used throughout this study. Virus assay was carried out by the plaquing method in monolayers of MDBK cells grown in 32 mm Petri dishes as described before (12). Plaquing was achieved using MEM supplemented with 0.5% specific anti-IBRV serum produced in rabbits (12).

Experimental Animals

Three 12 month old Jersey calves were used in the experiment. One calf (number 40) had been inoculated intranasally three months prior to the commencement of the experiment with 2 x 10^7 plaque forming units (PFU) or a field strain of IBRV.

The other two calves (numbers 37 and 49) apparently had no previous exposure to IBRV since their sera did not contain detectable levels of neutralizing antibody. Each calf was inoculated twice with 5 x 10^6 PFU of IBRV at a four-week interval. The calves were bled at eight days (early sample) and 21 days (late sample) after each inoculation. Serum was collected from the blood samples and stored at –20°C.

Immunoglobulins

The procedure for preparing 7S and 19S globulins from serum was described (12). Gel filtration in a Sephadex G200 column was used and pooled eluates of the globulin peaks were concentrated to the original volume of the serum applied to the column. Concentration was achieved by pressure dialysis against a balanced salt solution containing 1% FCS (BSS). Fetal calf serum was added to the 19S globulin preparations to reach a final concentration of 25% and these preparations were also treated with ultrasonic sound before storing at 4°C as described before (12).

Neutralization Kinetics

The neutralization kinetics procedure has been described (12). In this test 0.5 ml of IBRV, diluted in BSS to contain 2 x 10^4 PFU, was rapidly mixed with a suitable dilution of globulin and 0.1 ml of fresh guinea pig serum. The globulin dilution used was determined in preliminary experiments so that at this dilution approximately 80-90% of the virus was neutralized in 15 minutes. All reagents used in this test were prechilled in ice water. The influence of complement derived from guinea pig serum (C) on virus neutralization was determined by carrying out parallel tests using BSS instead of guinea pig serum. For the purpose of this study it was assumed that the enhancing effect of guinea pig serum on virus neutralization by specific antibody was due to complement since previous work has shown that this enhancing effect is due to the complement system (8). Immediately after mixing, a 0.1 ml sample was taken from the mixture and diluted 1:100 in chilled BSS. The rest of the mixture was incubated at 37°C in a waterbath. Three further samples were taken at five minute intervals and diluted as before. Virus assay was then carried out on the diluted samples. Each batch of guinea pig serum was tested for nonspecific antiviral activity by carrying out the neutralization kinetics procedure using FCS instead of anti-IBRV globulins.

The rate of virus neutralization was determined by plotting the log of the virus titer versus time (in minutes) and by calculation of the neutralization rate constant (K) as described by McBride (10). The K values allowed direct comparison between globulins since differences in the dilution of the globulins are compensated for. In addition, K ratios were calculated which represent the ratio of a K value obtained with a particular globulin in the presence of C to the K value obtained with the same globulin in the absence of C.

TABLE III. Neutralization Rate Constants (K) of Globulins Derived from Early Bovine Sera after a Second Exposure to IBRV

Calf	Globulin	K		K Ratio[b]
		Without C[a]	With C	
37	19S	0.03	0.78	25.0
49	19S	0.003	0.56	179.0
40	19S	0.12	1.73	15.0
37	7S	1.48	1.21	0.8
49	7S	2.24	2.73	1.22
40	7S	9.74	13.42	1.38

[a]Guinea pig complement
[b]Ratio of K value with C to K value without C

RESULTS

The results of the neutralization kinetics trials are given in Tables I to V.

The neutralization rate constants (K) provide a means whereby the neutralization capacity of globulins derived from the anti-IBRV calf sera could be compared. The value of the K ratio provides a measure of the enhancing effect C has on the neutralization of IBRV by a globulin preparation. It is clear from the tables and Fig. 1 that C markedly enhanced neutralization of IBRV when 19S AB preparations were used. In contrast, C did not seem to effect the rate at which IBRV was neutralized by 7S globulins (Fig. 2). Thus, when K values of the various globulins were compared the values obtained with 19S globulins in the presence of C and values ob-

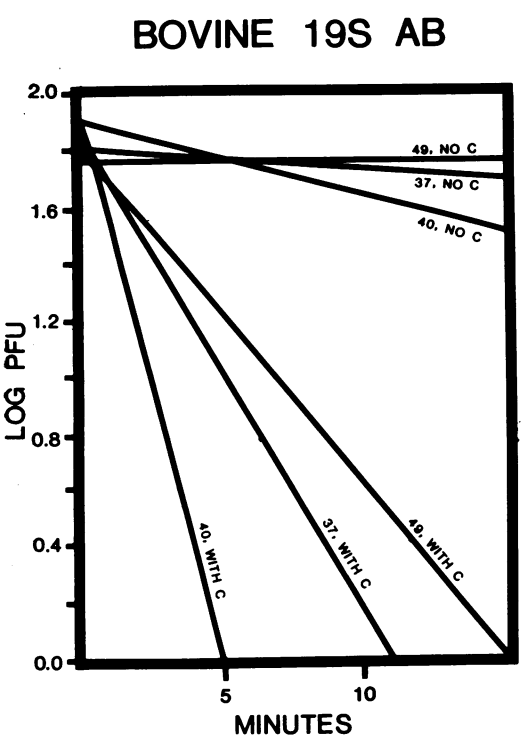

Fig. 1. The effect of complement (C) on the neutralization of IBRV by bovine 19S AB. The 19S globulins used were derived from sera collected from calves eight days after a second inoculation with IBRV. Note that C markedly enhanced the rate at which IBRV was neutralized by 19S AB.

tained with 7S globulins in the absence of C were used.

After primary inoculation of the calves with IBRV early sera contained only slight neutralizing activity in the 7S globulin fraction. However, virus neutralization was readily detected in the 19S globulin fraction (Table I). The results of the neutralization tests done with globulins from sera taken 12 days later (late sample) contrasted sharply with the results obtained with the early serum globulins. The 19S globulins from late sera of the calves after primary exposure to IBRV neutralized the virus poorly while 7S globulins from these sera rapidly neutralized IBRV (Table II).

The early sera obtained from calves after a second inoculation of IBRV once again contained readily detectable levels of neutralizing 19S AB. The K values increased from the approximate preexposure level of 0.1 to 0.6 and greater (Table III). However, the early sera collected after a second exposure to the virus contained on average an even greater amount of neutralizing activity in the 7S globulin fraction compared to the 19S fraction (Table III). One serum (40) had a 7S K value of almost 10.0 compared to the 1.73 K value of its 19S fraction. The K values of 7S AB from early sera after a second exposure to IBRV did, however, increase above the levels obtained just prior to the second inoculation.

Only one late serum collected after the second inoculation was tested. The 7S fraction from this serum contained a considerable amount of neutralizing activity but very little activity was detected in the 19S fraction (Table IV). The globulins derived from early serum of this calf after a third exposure to IBRV had neutralization rates similar to early sera after a second exposure to the virus. The K value of 19S AB increased markedly above the level obtained immediately prior to the third inoculation. The 7S AB from this serum neutralized IBRV very efficiently but its activity did not increase significantly after the third inoculation (Table V).

The fluctuation observed with the 19S

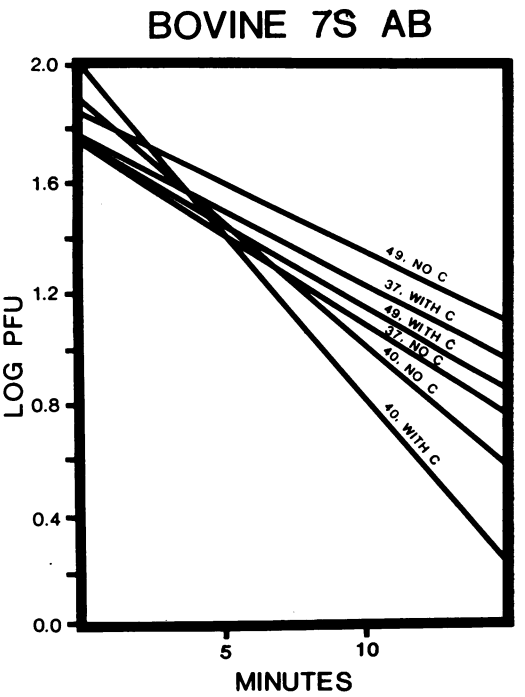

Fig. 2. The effect of complement (C) on the neutralization of IBRV by bovine 7S AB. The 7S globulins used were derived from sera collected from calves eight days after a second inoculation with IBRV. Note that C did not seem to enhance the rate at which the virus was neutralized.

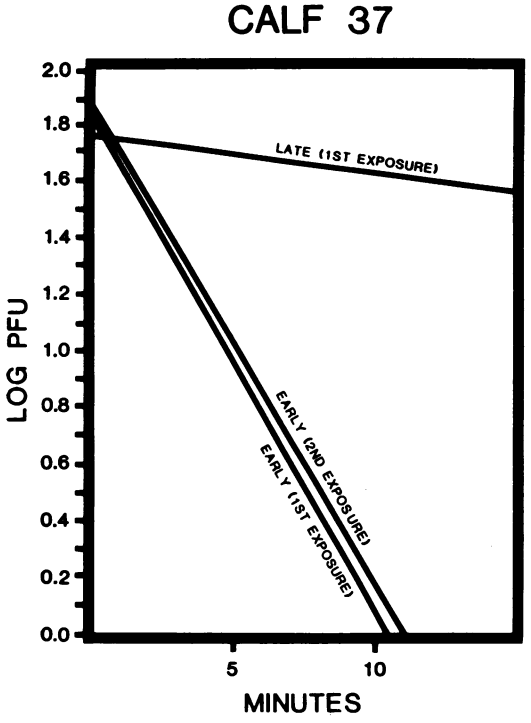

Fig. 3. The rate at which IBRV was neutralized by 19S globulins derived from the serum samples of calf 49. Complement was used in this test. Note that the 19S AB from the late serum sample did not neutralize the virus significantly while the AB from early sera rapidly neutralized the virus.

AB neutralizing activity can be seen in Figs. 3 to 5. It is clear that the 19S globulins from all the late sera tested contained little neutralizing activity while neutralizing activity was readily detected in all the 19S globulins from early sera.

DISCUSSION

In a previous study the capacity of rabbit immunoglobulins to neutralize IBRV in presence and absence of guinea pig complement was determined (12). However, the method of immunizing the rabbits differed somewhat from the procedure used in this study for cattle. The rabbits were hyperimmunized three times at three week intervals with 2-4 x 10^8 PFU IBRV which had first been partially purified and concentrated by differential centrifugation. In addition, the first two inoculations were administered with adjuvant (Freund's) while the last inoculation was given intravenously. It was found that 19S rabbit globulins and early 7S rabbit globulins required C for IBRV neutralization while neutralization of this virus with late 7S globulins was only very slightly enhanced by C.

In the work reported here it was established that bovine 19S anti-IBRV globulin required C for virus neutralization. In contrast, the 7S AB preparations were not enhanced by C. This work is in agreement with that reported previously for rabbit anti-IBRV globulins except that early rabbit antisera collected after a single inoculation with IBRV contained C-dependent 7S globulins (12). Since it has been shown that early 7S AB are probably directed against subsurface virion antigens (4) it is possible that levels of these antibodies

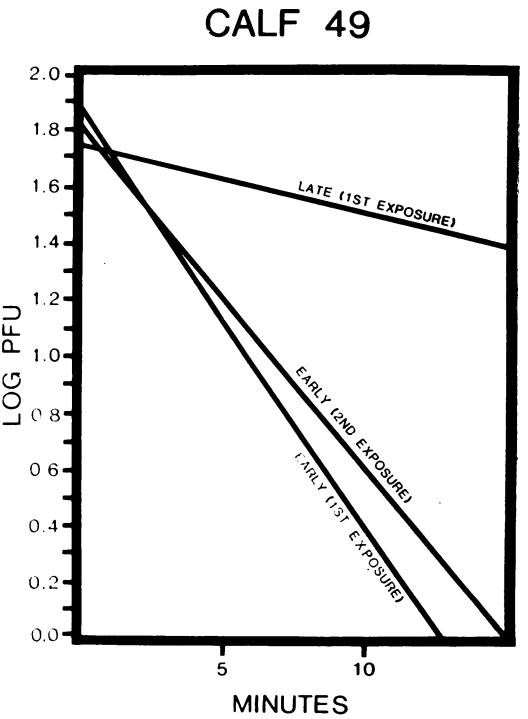

Fig. 4. The rate at which IBRV was neutralized by 19S globulins derived from the serum samples of calf 37. Complement was used in this test. Note that the 19S AB from the late serum sample did not neutralize the virus significantly while the AB from early sera rapidly neutralized the virus.

TABLE IV. Neutralization Rate Constants (K) of Globulins Derived from Late Bovine Sera after a Second Exposure to IBRV

Calf	Globulin	K Without C[a]	K With C	K Ratio[b]
40	19S	0.01	0.14	5.8
40	7S	5.66	5.84	1.03

[a]Guinea pig complement
[b]Ratio of K value with C to K value without C

TABLE V. Neutralization Rate Constants (K) of Globulins Derived from Early Bovine Antisera after a Third Exposure to IBRV

Calf	Globulin	K Without C[a]	K With C	K Ratio[b]
40	19A	0.10	0.51	5.0
40	7S	7.31	6.03	0.82

[a]Guinea pig complement
[b]Ratio of K value with C to K value without C

are perhaps very low in early bovine antisera. This may be explained by the relatively small dose of virus received by the calves compared to that inoculated into rabbits especially when the difference in body weight of the two animal species is considered. In addition, the rabbits were inoculated with a partialy purified virus suspension emulsified in an oil adjuvant which may have resulted in the rabbits being exposed to a greater proportion of degraded virions and since replication of IBRV probably does not occur in rabbits the type of immune response by the two species to IBRV may be quite different.

The C-dependence of 19S AB in virus neutralization has been observed with several other herpes viruses (3, 5, 15). Hampar *et al* (5) working with herpes simplex virus and Potgieter and Maré (12) working with IBRV reported that C slightly enhanced the rate at which these viruses were neutralized by late rabbit 7S AB. This phenomenon was not observed in the present study with bovine anti-IBRV 7S AB which may represent another difference between the immune response of rabbits and cattle.

Virtually all neutralizing activity of early bovine sera after primary exposure to IBRV was in the 19S globulin fraction since very little virus neutralization was observed with 7S globulins even in the presence of C. One would thus expect neutralization of early whole bovine antisera to be enhanced by C. In rabbits, however, the enhancing effect C has on virus neutralization by early whole serum after a primary immune response to IBRV is not only due to 19S AB but also to C-dependent 7S AB (12).

Complement dependency of neutralizing AB to IBRV is thus an indication of the presence of 19S AB in bovine sera. However, when bovine sera contained 7S AB it was present at a much higher concentration than 19S AB. Thus, in whole serum the presence of 19S AB is probably masked by the much higher concentrations of 7S AB. This masking of 19S C-dependent AB by 7S non C-dependent AB has been demonstrated in rabbit anti-IBRV antisera (12).

Several conclusions may possibly be drawn from this work. Firstly, sera from cattle that have had a recent IBRV infection probably would contain C-dependent 19S AB and secondly, the sera would contain little 7S AB if these animals had been exposed to the virus for the first time. In addition the presence of 7S AB in bovine

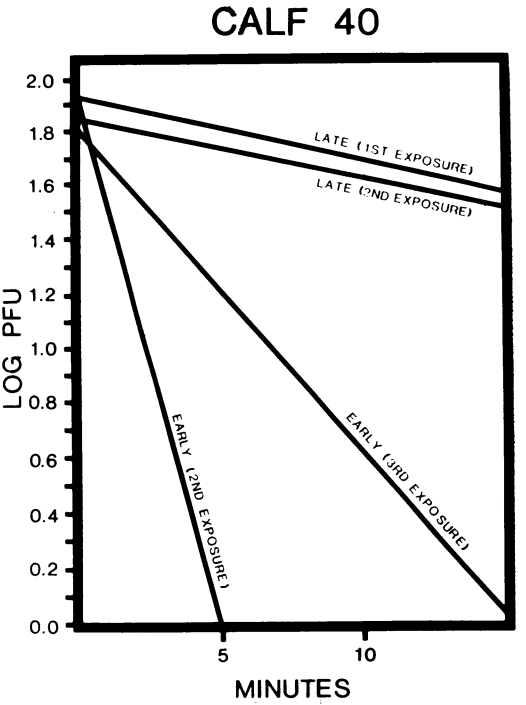

Fig. 5. The rate at which IBRV was neutralized by 19S globulins derived from the serum samples of calf 40. Complement was used in this test. Note that the 19S AB from the late serum samples did not neutralize the virus significantly while the AB from early sera rapidly neutralized the virus.

serum should indicate that some time has elapsed since the animal had been infected with IBRV and that the presence of relatively high levels of both 7S and 19S AB should indicate a reinfection by the virus and perhaps recrudescence of the disease.

It has become evident that IBRV may become latent in infected cattle (7, 14) and experimental reactivation of the virus in immune cattle after corticosteroid treatment has been demonstrated (14). In one study it was concluded that recrudescence of IBR and (or) reinfections of cattle by IBRV probably occurs under natural circumstances based on virus isolation, serology and clinical signs of respiratory illness (S. J. Hyland, B. C. Easterday and R. Pawlisch — personal communication). More conclusive epidemiological studies with IBRV infection in cattle could probably be made by studying the relative levels of 19S and 7S globulins and fluctuations of these AB in cattle. In addition, since it seems possible to determine the recent immunological history of IBRV in cattle by determining the relative 7S and 19S AB levels a diagnosis of IBRV infection may be made in the absence of paired serum samples.

ACKNOWLEDGMENTS

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BOOK REVIEW

AMPHIBIANS — GUIDELINES FOR THE BREEDING, CARE AND MANAGEMENT OF LABORATORY ANIMALS. *Published by the National Academy of Sciences, Washington, D.C. 1974. 153 pages. Price $5.25.*

A perusal of the tale of contents of this guideline indicates the care that has gone into the organization of the subject and attention given to important detail. If your need is for information on whether to or how to, you will find this book helpful. It is a most concise directory of helpful information, while at the same time indicating where the deficiencies in knowledge are. This, along with the other guidelines should be on the shelf of all animal facility directors and in the library of all institutions doing animal research. — *J. D. Schroder.*