Precipitin Response of Cattle to Bovine Papilloma Virus

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SUMMARY

Precipitins for bovine papilloma virus (BPV) which developed following experimental infection of 21 calves were characterized by diethylaminoethyl-cellulose chromatography, gel filtration with Sephadex G-200, sucrose density gradient, and analytic ultracentrifugation. A 19S antibody alone was found 1 week after initial exposure to BPV which persisted at demonstrable levels for 8 weeks. Both 19S and 7S antibodies were found between 2 and 8 weeks. A 7S antibody alone was consistently found in sera 16 weeks after the initial exposure to BPV. Precipitins appeared earlier than development of resistance to reinfection with BPV, and their presence in serum could not be correlated with growth or regression of the bovine fibropapillomas.

INTRODUCTION

A feature of cutaneous fibropapillomatosis (warts) in cattle is the spontaneous regression of the warts without treatment (21). Multiples warts on an individual animal usually regress simultaneously, but this occurs later than development of resistance to reinfection with BPV (16). Cattle do not develop as high a level of neutralizing antibodies in their sera following exposure to BPV as do horses similarly exposed (26). Antiviral neutralizing antibody has been demonstrated in serum from a natural case of bovine fibropapillomatosis using a tissue culture system (5). CF antibodies in bovine serum have been found after hyperimmunization with wart suspension in Freund's adjuvant (27), but Rosenberger et al. were unable to find precipitins for bovine papilloma virus in convalescent serum of cattle by the CF or precipitin test (24). A gel diffusion precipitin test with purified BPV as antigen has been developed (17).

MATERIALS AND METHODS

A suspension of BPV (isolate 301) was used as antigen, the preparation of which has been described previously (17).

Serums were obtained at successive intervals following exposure of 21 calves to BPV. These calves had received an i.v. injection of bovine papilloma suspension containing active virus, followed by at least 9 successive exposures to BPV on their skin, at intervals for 26 weeks, in a study of a development of resistance to reinfection. Details of the exposure of individual animals to BPV are given in a previous report (16). The various serum samples were examined in different procedures to characterize their antibody.

Serum fractions containing precipitins were separated by chromatography on a column of Sephadex G-200 with a modification of the procedure of Flodin et al. (10). Prior to use, Sephadex gels were soaked in distilled water for 48 hours with several exchanges of water and finally equilibrated. Layers of course Sephadex G-25 approximately 2 cm in thickness were placed under and above the column of Sephadex G-200. The total column was 2.5 x 56 cm, and the top was covered with filter paper. The column was equilibrated with buffer consisting of 0.1 M Tris-HCl (pH 8.0) in 2 M NaCl for 24 hours and the flow rate adjusted to 10 to 12 ml per hour. Serum samples (1.5 to 3 ml) were dialyzed against the Tris buffer for 24 hours at 4°C and then applied to the column. The effluent was collected in 2-ml portions with a fraction collector. Determinations of protein in the fractions were made with a Hitachi Perkin-Elmer spectrophotometer at a wavelength of 280 mμ. The column was further equilibrated with 0.1 M Tris-HCl (pH 8.0) in 2 M NaCl for 24 hours and the flow rate was 10 ml per 5 minutes. Each serum sample of 16 ml was dialyzed against the Tris buffer overnight at 4°C before it was placed on the column. The serum was eluted with 200 ml of each following phosphate buffers: I, 0.015 M, pH 6.3; II, 0.04 M, pH 6.0; III, 0.1 M, pH 5.8; IV, 0.3 M, pH 5.5; V, 0.4 M + 2 M NaCl, pH 4.3. Ten-ml fractions were collected and optical density at 280 mμ was used for localization of their protein content. The pooled fractions were concentrated approximately 10-fold by pervaporation in dialysis tubing.

A DEAE-cellulose (Carl Schleicher and Schnell Co., Keen, N. H.) column was prepared using the stepwise elution method of Sober and Peterson (28). The exchanger was equilibrated with 0.015 M phosphate buffer at pH 6.3, and after packing the column was further equilibrated with the same buffer for 24 hours. The flow rate was 10 ml per 5 minutes. Each serum sample of 16 ml was dialyzed against the 0.015 M phosphate buffer overnight at 4°C before it was placed on the column. The serum was eluted with 200 ml of each following phosphate buffers: I, 0.015 M, pH 6.3; II, 0.04 M, pH 6.0; III, 0.1 M, pH 5.8; IV, 0.3 M, pH 5.5; V, 0.4 M + 2 M NaCl, pH 4.3. Ten-ml fractions were collected and optical density at 280 mμ was used for localization of their protein content. The pooled fractions were concentrated approximately 10- to 20-fold and tested for antibody activity.

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2Abbreviations used are: BPV, bovine papilloma virus; CF, complement-fixing; DEAE, diethylaminoethyl; 2-ME, 2-mercaptoethanol.

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Density gradient centrifugation were done with the method of Tevethia (30). Gradients of sucrose were prepared in 5-ml cellulose nitrate tubes by layering successively 1.1-ml volumes of 40, 30, 20, and 10% sucrose and allowed to stand at 4°C for 24 hours. A half ml of serum sample was layered on top of the sucrose gradient and centrifuged for 18 hours at 35,000 rpm using an SW 25 rotor in a Beckman Spinco Model L ultracentrifuge. Ten to 12 samples of approximately 0.4 ml each were collected from the bottom of the tube.

The sedimentation velocities of the pooled fractions from DEAE-cellulose and Sephadex G-200 chromatography showing antibody activity were determined by centrifugation in a Beckman Spinco Model E analytic ultracentrifuge at 44,770 rpm and 59,780 rpm. Samples were thoroughly dialyzed against 0.14 M NaCl buffered with barbital to pH 8.0. The sedimentation coefficients were not corrected to standard conditions of 20°C in water.

Immunoelectrophoresis was carried out according to the micromodification of Scheidegger (25) using a gel of 1.0% ion agar No. 2 in pH 8.2 barbiturate-acetate buffer. Cellulose acetate electrophoresis was performed on Sepaphore III (Gelman Instrument Co., Ann Arbor, Michigan 48106) cellulose acetate membranes according to the procedures described by the manufacturer. The microdiffusion precipitin test and 2-ME treatment were performed according to methods described in detail (12, 17).

RESULTS

Precipitin was found in serums of 7 of the 21 calves one week after initial exposure and in 19 calves at 2 weeks (Table 1). All calves had precipitin at 8 weeks. The 2-ME-sensitive antibody was found in 7 calves at 1 week, 9 calves at 2 weeks, and only 2 calves at 4 weeks. The 2-ME-sensitive antibody was not demonstrable at 16 weeks. The 2-ME-resistant antibody appeared at 2 weeks and was found in all calves at 16 weeks.

Serums of 10 calves were selected for centrifugation in sucrose gradients to separate 7 S and 19 S antibodies. The 7 S antibodies were sedimented approximately one-third of the distance of gradient and the precipitin was not affected with 2-ME treatment. The 19 S antibodies were found at the bottom of the tube and precipitin activity was inactivated with 2-ME treatment. The 19 S antibody alone was demonstrable at 1 to 8 weeks and could not be detected at 16 weeks (Table 1). Both 19 S and 7 S antibodies were found at 2 to 8 weeks. At 16 weeks and later, 7 S antibody alone was demonstrable. Only 7 S antibody was found in 3 calves at 4 weeks and in all calves at 16 weeks.

Serums from Calves 261 and 267 were used in further attempts to characterize the precipitin. The serum proteins from Sephadex G-200 chromatography were separated into Peaks I, II, and III. The precipitin reaction of serum at 2 weeks from Calf 267 was found to be associated with fractions from Peak I (Chart 1). Treatment of this pool with 2-ME completely eliminated precipitin activity. The location of gamma-1M-globulin and alpha-globulin in Peak I was demonstrated by electrophoresis and immunoelectrophoresis. The serum bands with 2-ME treatment were submitted to analytical centrifugation, and 19 S antibody alone was demonstrable at 16 weeks (Calf 267) and 26 weeks (Calf 261) showed precipitin activity in Peak II, and the precipitin reaction persisted after treatment with 2-ME. Immunoelectrophoretic and electrophoretic analysis revealed 7 S gamma-globulins, alpha-globulins, beta-globulins, and traces of albumin.

Of the sera from Peak I of the Sephadex G-200 chromatography was submitted to analytical centrifugation, and protein sedimented as a single symmetrical peak with a coefficient of $S_{20,w} = 19.3$ at 44,770 rpm. Pooled fractions of Peak I of DEAE-cellulose chromatography had a protein with sedimentation coefficient of $S_{20,w} = 6.8$ at 59,780 rpm.

<table>
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<th>Susceptibility and antibodies</th>
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<td>Susceptibility of 21 calves</td>
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<td>Partial resistance</td>
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<td>7 S antibody</td>
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Correlation of development of precipitins and resistance to successive intradermal injections of 10 percent bovine papilloma suspension in calves.

Table 1

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Chart 1. Sephadex G-200 column chromatogram of serum from Calf 267 at 2 weeks after inoculation with bovine papilloma virus. Viral precipitins were found in fractions from black area.

A single precipitin line in gel diffusion test with BPV antigen was developed by the active fractions from Sephadex G-200 and DEAE-cellulose chromatography (Fig. 3). The same precipitin patterns were formed by whole serums and fractions from sucrose gradient density centrifugation (Fig. 3).

The data in Table 1 concerning susceptibility and resistance to successive injection of bovine papilloma suspension (BPS) are abbreviated from a previous study (16). When calves were susceptible the warts grew progressively to a large size and persisted longer. Warts produced when the calves were partially resistant grew a relatively short time and were small. No warts developed when the calves were resistant to the injection of BPV. Precipitins developed in most calves earlier than resistance to reinfection with BPV. Resistance to successive BPV challenge developed progressively from 2 to 16 weeks after injection and 7 S antibody also developed during the same period (Table 1). However, some calves were still susceptible to BPV after 7 S antibody was formed. The warts at early inoculation sites showed continuous growth after the calves had developed 7 S antibodies and resistance to BPV infection.

DISCUSSION

In these experiments BPV precipitins with different physicochemical and immunologic properties were found to be associated with different time intervals after BPV infection. The first antibody demonstrable after BPV infection was a macroglobulin. DEAE-cellulose and Sephadex G-200 chromatography indicated this precipitin as 19 S (6, 10, 12, 14). The results of 2-ME treatment and immunoelctrophoretic analysis indicated it to be similar to gamma-1M-globulin of bovine serum (19). Sucrose gradient centrifugation and analytic ultracentrifugation experiments also indicated that the antibody was 19 S (4, 12, 19). The 19 S antibody was demonstrable at 1 week postinoculation; it was found later in all calves and had disappeared at 16 weeks after their first exposure to BPV.

The antibody which appeared later was eluted in the position of 7 S globulin with DEAE-cellulose and Sephadex G-200 chromatography (7, 10). The position in sucrose density gradient centrifugation and sedimentation coefficient of this antibody was approximately to that of 7 S globulin (4, 9, 12, 30). The antibody in whole serums as well as the purified antibody was found to be resistant to inactivation by 2-ME, which indicated it to be 7 S antibody (4, 9, 12). Analysis revealed the same electrophoretic mobility as 7 S gamma-1- and 7 S gamma-2-globulin of bovine serum (8, 20). The early antibody response of calves was 19 S antibody alone or both 19 S and 7 S antibodies. The 7 S antibody was detectable soon after or simultaneously with the appearance of 19 S antibody. The 7 S antibody alone was found in some calves from 4 to 16 weeks after exposure, and later all calves showed 7 S antibodies.

A precipitin of human wart virus was found by the gel diffusion test in serums of about half of 42 patients with warts (1). The examination of 18 positive anti-wart serums revealed that 13 contained only 19 S antibody, 3 contained 19 S and 7 S antibody, and 2 contained 7 S antibody only (11). The 19 S antibody alone was found in patients up to 6 months after they first came to the clinic. Goffe et al. (11) suggested that the common recurrence of warts in man might be due to lack of 7 S antibody.

In contrast to the observations in papillomatosis of man (11), all experimental calves had only 7 S antibody at 16 weeks after the initial injection with BPV. The experimentally inoculated calves had multiple warts, whereas in humans warts are usually few in number and localized. The development of multiple warts might release sufficient BPV antigen to have elicited 7 S antibody in all the experimental calves.
Little is known about the immune mechanism responsible for the regression of bovine fibropapillomas. Serum therapy for bovine papillomatosis has not been effective (24). Wart tissue vaccine has been used therapeutically; however, the results are variable (2, 22, 23, 29). The results of this study indicate that development of precipitin for BPV was not responsible for papilloma regression but could be correlated with resistance to reinfection with BPV.

REFERENCES


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