IDENTIFICATION AND CHARACTERIZATION OF DIFFERENTIATING SOLUBLE ANTIGENS OF SHEEP AND GOAT POXVIRUSES

T.V.S. RAO[1], B.S. NEGI[1]*, M.P. BANSAL[2]

[1]Pox Virus Diseases Laboratory, Division of Virology, and [2]Immunology Section, Indian Veterinary Research Institute, Mukteswar-Kumaon 263 138, India

Received July 9, 1996

Summary. – Soluble antigens of sheep and goat poxviruses (SPV, GPV) were isolated and purified from scab suspensions prepared from lesions of experimentally infected homologous hosts. The soluble antigens were then subjected to sequential ammonium sulphate precipitation. All the obtained fractions reacted in counter immunoelectrophoresis (CIE) with both the antisera against SPV and GPV except the fraction obtained at 30% saturation level (30% SSPV), which did not react with antiserum against GPV. This differentiating soluble SPV antigen was found to consist of 210 K proteins in exclusion chromatography. The 210 K proteins contained 3 polypeptides of 100, 35 and 17 K in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). The study thus gave an evidence that the SPV-specific proteins are of a higher molecular mass nature.

Key words: sheep poxvirus; goat poxvirus; soluble antigens; differentiating antigen; exclusion chromatography; SDS-PAGE

Introduction

Sheep pox and goat pox are a few of the most severe pox infections of domestic animals. The cutaneous lesions of these infected animals have specific precipitinogens which give precipitation reaction with specific antibody against capripoxviruses (Sambyal and Singh, 1978). These precipitinogens are known as "soluble antigens", which are released or can be separated from the infectious viral particles.

The diagnosis of pox infections in sheep and goats is usually made on clinical grounds, but confirmed by serological tests. Although many laboratory tests like agar gel precipitation (Pandey and Singh, 1972), CIE (Sharma *et al.*, 1988a; Puranchand *et al.*, 1985), enzyme-linked immunosorbent assay (ELISA, Sharma *et al.*, 1988b; Datta and Soman, 1990), latex agglutination (Rao *et al.*, 1996) and spot agglutination (Tiwari *et al.*, 1996) have been developed, none of them is useful for differentiation of goat pox from sheep pox infection since these are closely related. In order to differentiate them, there is a need to identify virus-specific antigens so that they can be further exploited for formulating differentiation kits or reagents. Hence, the present study was conducted to identify and characterize differentiating polypetides among the soluble SPV and GPV antigens.

*Corresponding author.

Abbreviations: CIE = counter immunoelectrophoresis; ELISA = enzyme-linked immunosorbent assay; GPV = goat poxvirus; PBS = phosphate-buffered saline; SDS-PAGE = polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate; SPV = sheep poxvirus; 30% SSPV = soluble SPV antigen fraction obtained at 30% salt saturation

Materials and Methods

Animals. Apparently healthy non-descript sheep and goats of either sex, about one-year-old, and having no history of pox infections, were used in this study.

Viruses. The Jaipur strain of SPV and the Sambalpur strain of GPV maintained by periodical skin-to-skin transfers in homologous hosts were used for experimental infections.

Soluble antigens were obtained by ultracentrifugation of skin scab suspensions prepared from the lesions of experimentally infected sheep and goats (Rao *et al.*, 1996).

Hyperimmunization of rabbits against healthy sheep/goat skin extracts. The healthy skin extracts were partially purified by ultracentrifugation similar to that described above for the preparation of soluble antigens. The antisera were raised (Hudson and Hay, 1989) by giving four injections of healthy skin proteins (200 µg per dose), the first (intramuscular) with complete Freund's adjuvant, the second and third (intramuscular) with incomplete Freund's adjuvant, and the last (intravenous) without any adjuvant, on days 0, 14, 21 and 35. Ten days after the last injection, the sera were collected and stored at -20°C.

Purification of rabbit IgG by DEAE-cellulose chromatography. The method described by Dubey (1983) was followed using 0.01 mol/l phosphate buffer pH 8.0 for elution.

Coupling of rabbit IgG to Sepharose CL-4B. Sepharose beads (Pharmacia, USA) were activated by cyanogen bromide at alkaline pH in a fume hood (Hudson and Hay, 1989). Then, 50 mg of IgG (10 mg/ml) was added to the activated beads and the mixture was stirred overnight at 4°C. The beads were subsequently loaded into a 20 ml plastic syringe in which a piece of glass wool was applied. After washing with phosphate-buffered saline pH 7.2 (PBS), the beads were suspended in 1 mol/l glycine pH 8.0 at 4°C for 6 hrs, and finally washed with PBS before use.

Purification of soluble SPV/GPV antigens. The concentrated soluble antigens were purified by passing them repeatedly through column of cyanogen bromide-activated Sepharose coupled with rabbit IgG using PBS as elution buffer. The elution of bound proteins was done using 0.1 mol/l glycine-HCl buffer pH 2.5. The fractions of purified soluble SPV/GPV antigens thus obtained in PBS were tested for reactivity in CIE using heterologous antiserum raised against healthy sheep/goat skin extracts.

Antisera against SPV/GPV were raised separately in healthy sheep and goats (2 in each case) by giving four injections of the respective purified soluble antigen (2, 3, 4, and 5 mg per dose, respectively). The first injection (intramuscular and subsutaneous) with complete Freund's adjuvant, the second and third (intramuscular and subcutaneous) with incomplete Freund's adjuvant and the last (intravenous) without any adjuvant, were given on days 0, 21, 28 and 42. Ten days after the last injection, the sera were collected and Igs were isolated by 3 times repeated salting out at 40% ammonium sulphate saturation (Dubey, 1983).

Fractionation of soluble SPV/GPV antigens by sequential ammonium sulphate precipitation was carried out at 30, 60 and 90% saturation levels. The precipitates, after resuspension in and dialysis against saline, were checked for specific SPV/GPV antigens by CIE using the respective antisera.

CIE was carried out according to Rao *et al.* (1996).

Controls. The sera collected from sheep and goats (six each) experimentally infected with SPV and GPV, respectively, and the sera from apparently healthy uninfected animals (six each) were tested in CIE for the presence of specific antibodies using the 30% SSPV.

Exclusion chromatography on Sephadex G-200 column was done according to Hudson and Hay (1989). Briefly, the column was packed with Sephadex G-200 (Sigma, USA), equilibrated with PBS (packed column dimensions 30 x 2.5 cm), and subsequently calibrated with the proteins of known molecular mass. The 30% SSPV was charged onto the calibrated column and eluted in 2.6 ml fractions. Their A_{280} was recorded. The molecular mass of the 30% SSPV was determined according to Andrews (1964).

SDS-PAGE. After boiling for 5 mins in the presence of 2% ß-mercaptoethanol, the 30% SSPV was electrophoresed using the discontinuous system devised by Laemmli (1970) with 10% resolving gel and 5% stacking gel.

Results

The soluble SPV/GPV antigens were found to be free of healthy skin proteins as revealed in CIE using antisera raised against healthy skin extracts. All the antigen precipitates showed precipitation lines in both the homologous and heterologous systems with Igs of the antisera raised against soluble antigens. The results are presented in Table 1. However, the 30% SSPV was found to be reactive only with the hyperimmune serum against soluble SPV antigen, but not with that against soluble GPV antigen.

In testing various sera from sheep and goats with the 30% SSPV in CIE, the precipitation lines could be observed only with the sera collected from experimental sheep infected with SPV, while all the other sera were found to be negative.

Exclusion chromatography on Sephadex G-200 of the 30% SSPV showed only one peak of about 210 K while SDS-PAGE showed 3 polypeptides of 100, 35 and 17 K (data not shown).

Table 1. Reactivity of soluble SPV and GPV antigens in CIE

Ammonium sulphate fractions	Antiserum[a] against	
	soluble SPV	soluble GPV
Soluble SPV antigens		
30% saturation	+	–
60% saturation	++	++
90% saturation	+	+
Soluble GPV antigens		
30% saturation	+	+
60% saturation	+	++
90% saturation	+	+

[a]Ig fraction was used.
(–) = no precipitation lines; (+) = one precipitaion line. (++) = two precipitation lines.

Discussion

Soluble antigens were successfully isolated from infectious virus particles by ultracentrifugation and found to be non-infectious and precipitating (Isloor and Negi, 1995; Rao *et al.*, 1996). Some of them were identified as structural components of virus particles and efficiently employed in various serological tests (Sharma *et al.*, 1988a; Tiwari *et al.*, 1996). SPV and GPV are antigenically related by sharing only a few soluble antigenic components (Pandey and Singh, 1972; Subbarao *et al.*, 1984). Although other components of precipitinogens may not be common to both the viruses, it is often difficult to make a differential diagnosis based simply on the pattern of precipitation lines. However, the differential diagnosis of sheep pox and goat pox was made successfuly by extensive adsorption of the antisera (Datta and Soman, 1990), although these were closely related.

Here, the presence of reactivity in all the antigen precipitates revealed that almost all the antigens were responsible for the production of antibodies, provided the antisera were raised in homologous hosts using the adjuvant. This is in agreement with the earlier findings (Pandey and Singh, 1972; Isloor and Negi, 1995). The crossreactivity of these antisera with whole soluble SPV and GPV antigens further confirmed the fact that these two viruses were closely related (Kitching and Taylor, 1985; Sinha and Soman, 1987).

However, the absence of reactivity of the 30% SSPV with hyperimmune serum against soluble GPV antigen was probably due to a lack of specific antibody. This in turn indicated the absence of similar polypeptide(s) in the soluble GPV antigen and hence no antibody production. These results support the view that only a partial sharing of antigens exists between SPV and GPV (Bhambani and Krishnamurthy, 1963; Sharma nad Dhanda, 1969; Pandey and Singh, 1972; Subbarao and Malik, 1983). The specificity of the 30% SSPV was further indicated by the fact that this fraction was able to detect specific antibody in the experimental sheep infected with SPV, but not in the goats infected with GPV.

Thus, the behaviour of this differentiating soluble SPV antigen (precipitation at 30% salt saturation along with the exclusion chromatography behaviour) revealed its high molecular mass nature. Furthermore, the existence of a polypeptide band above 90 K in the soluble SPV antigen unlike in that of GPV (Isloor and Negi, 1995) confirmed the presence of specific polypeptides in SPV-infected animals. These results suggest that the SPV-specific proteins are of a higher molecular mass.

Hence, a detailed characterization of SPV-specific polypeptides with a large number of convalescent and experimental sera from capripox-infected animals, carried out in the future, could form an initiative to formulate certain simple, easy-to-prepare kits or reagents for differentiation of the two closely related diseases, sheep pox and goat pox, and might also reveal a possible emergence of virus variation. It would further help the researchers, clinicians and farmers in adopting precise control measures to prevent or reduce the occurrence of these devastating diseases.

Acknowledgements. We are thankful to the Director of the Indian Veterinary Research Institute, Mukteswar-Kumaon, for providing necessary facilities required to carry out this work, and to the staff members of the Pox Virus Diseases Laboratory for their help and cooperation.

References

Andrews P (1964): Estimation of the molecular weights of proteins by Sephadex gel filtration. *Biochem. J.* **91**, 222.

Bhambani BD, Krishnamurthy D (1963): An immunodiffusion test for the diagnosis of sheep and goat pox. *J. Comp. Path.* **73**, 349–357.

Datta S, Soman JP (1990): Dot-immunobinding technique in differential diagnosis of pox diseases of sheep and goats. *Indian J. Anim. Sci.* **60**, 1186–1187.

Dubey SK (1983): Purification of immunoglobulins of different classes. In Talwar GP (Ed.): *A Handbook of Practical Immunology.* Vikas Publishing House Pvt Ltd, New Delhi, pp. 71–83.

Hudson L, Hay FC (1989): *Practical Immunology.* 3Edn., Blackwell Scientific Publications, Oxford-London-Edinburgh-Boston-Melbourne, pp. 257–258.

Isloor S, Negi BS (1995): Characterization of soluble antigens of goat pox virus. *Indian J. Anim. Sci.* **65**, 240–245.

Kitching RP, Taylor WP (1985): Clinical and antigenic relationship between isolates of sheep and goat pox viruses. *Trop. Anim. Hlth. Prod.* **17**, 64–74.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Pandey R, Singh IP (1972): Soluble antigens of sheep and goat pox viruses as determined by immunodiffusion in agar gel. *Acta Virol.* **16**, 41–46.

Puranchand, Rao VDP, Garg SK, Singh IP, Chandra R (1985): Counter immunoelectrophoresis test for rapid diagnosis of sheep pox. *Brit. Vet. J.* **141**, 124–128.

Rao TVS, Negi BS, Dhar P, Asgola D (1996): Evaluation of latex agglutination test for the rapid detection of goat poxvirus antigen and antibodies. *Acta Virol.* **40**, 95–97.

Sambyal DS, Singh IP (1978): Immunogenecity of soluble antigens of sheep pox virus. *Indian J. Anim. Sci.* **48**, 511–514.

Sharma SN, Dhanda MR (1969): Serological studies on sheep and goat poxviruses. *Indian J. Anim. Sci.* **40**, 522.

Sharma B, Negi BS, Pandey AB, Bandyopadhyay SK, Shankar H, Yadav MP (1988a): Detection of goat pox antigen and antibody by the CIE test. *Trop. Anim. Hlth. Prod.* **20**, 109–113.

Sharma B, Negi BS, Yadav MP, Shankar H, Pandey AB (1988b): Application of ELISA in the detection of goat pox antigen and antibodies. *Acta Virol.* **32**, 65–69.

Sinha OP, Soman JP (1987): Comparative evaluation of some immunologic techniques for the diagnosis of pox infections of sheep and goats. *Indian J. Virol.* **3**, 61–70.

Subbarao MV, Malik BS (1983): Application of electro-immunodiffusion test for detection of antigenic relationship between sheep pox and goat pox viruses. *Acta Virol.* **27**, 177–179.

Subbarao MV, Malik BS, Sharma SN (1984): Antigenic relationship among sheep pox, goat pox and CPD viruses. *Acta Virol.* **28**, 380–387.

Tiwari AK, Rao TVS, Negi BS (1996): Spot agglutination test for the rapid diagnosis of goat pox. *Trop. Anim. Hlth. Prod.* **28**, 213–215.