Indian Journal of Animal Sciences **80** (8): 707–710, August 2010

Detection and characterization of soluble proteins of sheep pox virus

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Received: 23 September 2009; Accepted: 22 April 2010

ABSTRACT

Soluble proteins of sheep pox virus produced in lamb testis (LT) cells were investigated using graded precipitation of the virus-free culture supernatants with saturated ammonium sulphate solution. The proteins were separated from infected culture supernatants at 45% (A), between 45 and 60% (B) and from 60 to 80% (C) saturation of ammonium sulphate. SDS-PAGE and immunoblot analyses of the soluble proteins revealed a total of 33 and 24 proteins, respectively, with molecular weights ranging from 14 to 181 kDa. Of the 33 soluble proteins, 15 proteins in preparation A, 30 in B and 28 in C were detected in SDS-PAGE, whereas 6 proteins in preparation A, 21 in B and 18 in C showed reaction in immunoblotting. In SDS-PAGE of partially purified virus preparation (PPV) 18 proteins were detected. Of these, 8 were common with the proteins present in soluble protein preparations (A/B/C). These soluble proteins could be used as diagnostic antigen.

Key words: Capripoxvirus, Immunoblotting, SDS-PAGE, Sheep pox virus, Soluble proteins

Prevention and control of the sheep pox virus can be effected by vaccination of flocks with a live attenuated and/or killed vaccine. Nevertheless, breakdown in immunity has been reported despite the use of these vaccines (Batra *et al*. 1996, Malik *et al*. 1997). A recombinant protein (an envelope protein of the virus) used as a sub-unit vaccine protected the vaccinated experimental animals against virulent virus challenge (Carn *et al*. 1994 a, b). The soluble proteins of sheep pox virus induced protective neutralizing antibodies (Sambyal and Singh 1978). The limited information on these viral proteins is mainly based on immunodiffusion and immunoelectrophoresis (Sambyal and Singh 1978, Pandey and Singh 1982, Chand *et al*. 1985). However, the soluble proteins have not been fully characterized. The present study demonstrates the different protein patterns obtained by differential ammonium sulphate precipitation followed by SDS-PAGE and immunoblotting of sheep pox virus-infected cell lysates.

MATERIALS AND METHODS

Isolation of virus soluble proteins: For isolation of sheep pox virus-soluble proteins, lamb testis cell monolayer (LT cells) were grown in plastic tissue culture flasks (Nunc 175 cm²) using growth medium supplemented with 10% foetal calf serum as described by Plowright and Ferris (1958). A virulent sheep pox virus isolated in our laboratory from a field outbreak was used at the eighth passage level (Malik and Chand 1998). The proteins were separated from the supernatant of the virus-infected LT cells. When approximately 90% of the cells showed cytopathic changes, the infected cells were harvested using a rubber policeman and subjected to 2 cycles of quick freezing and thawing. Subsequently this was centrifuged at 1500 × g for 30 min at 4ºC. The supernatant obtained was centrifuged again at 90 000 × g for 1 h to pellet the virus. The supernatant was used to separate soluble proteins and the pellet after dissolving in NSS was loaded on a 5 ml cushion of 40% sucrose and centrifuged at 90 000 g for 1 h and used as partially purified virus (PPV) preparation in SDS-PAGE and immunoblotting.

Precipitation of proteins: The soluble proteins in supernatant were precipitated by saturated ammonium sulphate solution as described by Sambrook *et al*. (1989). At 45% saturation, the suspension was centrifuged and the pellet was saved while the supernatant was re-precipitated by raising the saturation of ammonium sulphate up to 60% and the turbid solution was again centrifuged at 1 500 × g for 30 min. The pellet was saved and finally the supernatant was precipitated at 80% saturation and was centrifuged as described above. In this way 3 pellets (obtained by 45%, between 45 and 60% and 60 and 80% saturation) of

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precipitated soluble proteins were obtained and designated as preparations A, B, and C respectively. All the preparations were dialyzed against PBS (pH 7.4). Subsequently the preparations were centrifuged at 2 000 × g for 30 min and the supernatants were used as soluble protein preparations. These were stored at –20ºC till use. These preparations of soluble proteins were analyzed by SDS-PAGE and immunoblotting. In addition, the PPV preparation and uninfected LT cells lysate were included in the study as controls. Both hyperimmune and convalescent sera were used to detect the sheep pox virus-soluble proteins by immunoblotting. Convalescent serum was obtained from a severely affected sheep during a field outbreak of sheep pox. Hyperimmune serum was raised in sheep according to the immunization schedule described by Chand *et al*. (1985).

SDS-polyacrylamide gel electrophoresis and Immunoblotting: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed (Chand *et al*. 1994) using the discontinuous system of Laemmli (1970). The molecular weights of different bands were estimated by following the protocols of Weber and Osborn (1969). The PPV and soluble protein preparations separated by SDS-PAGE were electrophoretically blotted onto the nitrocellulose membranes (NCM, 0.45 µm pore size) for 1.5 h at 0.8 mA/cm² of gel using a semi-dry method (Kyshse-Andersen 1984). Immunoblotting was done as described by Chand *et al*. (1994).

RESULTS AND DISCUSSION

In SDS-PAGE, 15 proteins ranging from 17 to 156 kDa were detected in preparation A and 5 proteins (Table 1) out of these reacted on immunoblot with sheep pox virus hyperimmune serum, whereas only 2 proteins (28 and 56 kDa) reacted on immunoblot when convalescent serum was used (Fig. 1). This observation indicated that 3 additional proteins reacted with hyperimmune serum. This difference was seen only in preparation A. One protein of 156 kDa was detected only in preparation A and appears to be precipitated completely at 45% ammonium sulphate saturation, as it was not detected in any other soluble protein preparations (B/C). As shown in Fig. 1, on immunoblot 2 proteins (28 and 35 kDa) detected in preparation A, were also detected in PPV. In preparation B, a maximum number of soluble proteins (30) of molecular weights ranging from 14 to 181 kDa were resolved in SDS-PAGE. Of these, 21 proteins reacted in immunoblotting with sheep pox virus hyperimmune serum. Similar numbers of proteins were detected using both hyperimmune and convalescent anti-sheep pox sera. Two proteins of 121 and 90 kDa were detected only by immunoblotting but not by SDS-PAGE. This may be due to greater sensitivity of immunoblotting as compared to SDS-PAGE. In this preparation 90 kDa protein was completely precipitated at 60% saturation of ammonium sulphate as it was not detected in preparation C. In SDS-PAGE, at position 60–70 kDa, a thick band was observed which on immunoblotting resolved in three discrete bands (Fig.1, Table 1). In preparation C, a total of 28 proteins ranging from 14 to 181 kDa were resolved on SDS-PAGE. Of these, 18 showed reaction by immunoblotting (Table 1). One protein of 121 kDa of preparation B was detected in immunoblotting but not in SDS-PAGE. In preparation C also, at position 60–70 kDa, a thick band appeared which was indistinguishable on SDS-PAGE but resolved into 3 discrete bands by immunoblotting. Thus, in total, 33 soluble proteins were detected in SDS-PAGE, ranging from 14 to 181 kDa. For comparison, the partially purified virus preparation (PPV) was analyzed along with soluble proteins. In this preparation, 18 proteins ranging from 14 to 98 kDa were detected by SDS-PAGE. Of these, 12 reacted in immunoblotting with both hyperimmune and convalescent sheep pox virus antisera (Table 1 and Fig. 1). In the PPV preparation there were 8 proteins which were common with all the 3 preparations of soluble proteins. The remaining 4 proteins, which were detected in PPV preparations only appear to be synthesized by the virus in such amounts that all the proteins are incorporated into the virus particle and are thus not detected in soluble protein preparation.

Graded ammonium sulphate precipitation of soluble proteins revealed that a maximum (30) of proteins was precipitated between 45 and 60% saturation, whereas no additional protein was found to be precipitated when ammonium sulphate saturation was raised from 60 to 80%. Analysis of soluble proteins of sheep pox virus revealed that of the 33 soluble proteins detected on SDS-PAGE, 24 were exposed to the immune system resulting in antibody response

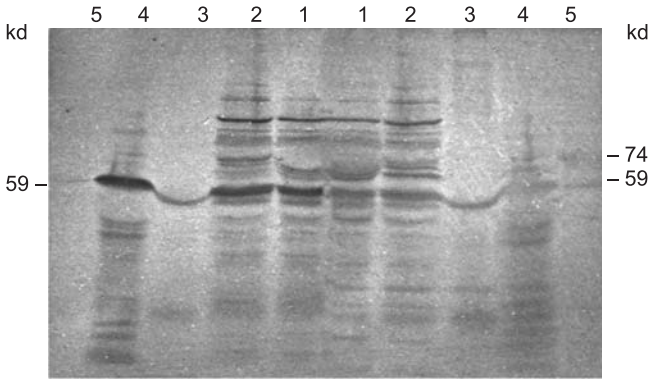

Fig. 1. Comparison of hyperimmune serum and convalescent serum in detection of soluble proteins of sheep pox virus by immunoblotting. Convalescent serum: Lanes–1. Preparation C, showing 18 bands; 2. Preparation B, showing 21 bands; 3. Preparation A, showing 2 bands; 4. Partially purified virus showing 12 bands; 5. Mock infected lamb testis cell lysate; Hyper immune serum: Lanes–1. Preparation C, showing 18 bands; 2. Preparation B, showing 21 bands; 3. Preparation A, showing 6 bands; 4. Partially purified virus showing 12 bands; 5. Mock infected lamb testis cell lysate.

Table 1. Proteins of sheep pox virus detected by SDS-PAGE and immunoblotting in different soluble preparations (A, B, and C) and partially purified virus (PPV)

S. No.	Proteins with molecular weights (kDa) in different preparations							
	PPV		A		B		C	
	SDS-PAGE	Immunoblot	SDS-PAGE	Immunoblot	SDS-PAGE	Immunoblot	SDS-PAGE	Immunoblot
1	–	–	–	–	181	–	181	–
2	–	–	–	–	175	–	175	–
3	–	–	–	–	166	–	166	–
4	–	–	156	156	–	–	–	–
5	–	–	147	–	147	–	147	–
6	–	–	–	–	140	–	–	–
7	–	–	131	–	131	131	131	131
8	–	–	124	–	124	124	–	–
9	–	–	–	–	–	121	–	–
10	–	–	113	113	113	–	113	–
11	–	–	–	–	107	–	107	–
12	98	–	–	–	98	98	98	98
13	–	–	–	–	–	90		
14	85	85	85	–	85	85	85	85
15	–	–	–	–	80	80	80	80
16	75	75	–	–	–	–		
17	–	–	**67**	–	**67**	67	**67**	67
18	65	65	**65**	–	**65**	65	**65**	65
19	–	–	**62**	–	**62**	62	**62**	62
20	59	59	–	–	–	–	–	–
21	–	–	56	56	56	56	56	56
22	–	–	–	–	52	52	52	52
23	–	–	–	–	46	46	46	46
24	44	44	–	–	–	–	–	–
25	–	–	–	–	43	43	43	43
26	42	42	–	–	–			
27	–	–	41	41	41	41	41	41
28	40	–	–	–	40	40	40	40
29	38	–	–	–	38	38	38	38
30	35	35	35	35	35	35	35	35
31	32	32	–	–	32	32	32	32
32	30	–	30	–	30	–	30	–
33	28	28	–	28	28	28	28	28
34	25	–	–	–	–			
35	23	23	23	–	23	–	23	–
36	19	19	–	–	19	–	19	–
37	17	–	17	–	17	–	17	–
38	14	14	–	–	14	14	14	14
Total	18	12	15	6	30	21	28	18

Proteins present in the bold were considered to be 3 in number in SDS-PAGE.

as evident by reaction in immunoblotting (Fig. 1). The remaining 9 proteins which did not react in immunoblotting are probably hidden and do not induce immune response or may be that the cell culture contaminated proteins. In the present study both hyperimmune and convalescent sera produced a different pattern of reaction in immunoblotting (Fig. 1).

Variation in the numbers of precipitation line due to variation in quality of antiserum has been observed by several workers (Uppal and Nilkantan 1967, Pandey and Singh 1972). Of the 33 soluble proteins, the hyperimmune serum reacted with 24 proteins and convalescent serum with 20 proteins in preparation A by immunoblotting. These results indicate that hyperimmune serum is comparatively better for detection of sheep pox virus-soluble proteins by immunoblotting. The normal control sheep serum did not produce any reaction in immunoblotting in both soluble as well as PPV preparations indicating that the hyperimmune and convalescent sera

reacted specifically with the viral proteins. In the present study an antigenic protein of 32 kDa was detected in PPV preparation and also in soluble protein preparations B and C. It has been demonstrated earlier that a 32 kDa envelope protein of the KS-1 isolate of sheep pox virus induces neutralizing antibodies and contains both B and T cell epitopes (Chand 1992). On the basis of similar electrophoretic mobility in SDS-PAGE, reaction in immunoblot by anti-sheep pox serum and its presence in PPV preparations, it appears that the 32 kDa protein detected in the present study is similar to the 32 kDa protein detected in KS-I isolate of capripox virus. However, additional molecular studies are required to confirm this assumption.

Present results indicate that sheep pox virus induces synthesis of a large number of structural and non-structural proteins, many of which are soluble and produce an antibody response in the natural host. The majority of these soluble proteins can be separated from infected LT cell lysate by differential ammonium sulphate precipitation. This study also provided more detailed understanding of the molecular mass and antibody response in natural infection and perceptibility at different concentration of ammonium sulphate which was not possible by the conventional methods as immunodiffusion or immunoelectrophoresis used earlier.

ACKNOWLEDGEMENTS

Authors are grateful to the CCS Haryana Agricultural University, Department of Veterinary Microbiology, Centre of Advanced Studies for providing necessary facilities and Dr S.K. Batra, Incharge, Pox Virus Laboratory for their untiring help and co-operation. The authors are grateful to the Indian Council of Agricultural Research, New Delhi, for providing financial assistance to carry out this work. Help received from Dr Gaya Prasad, Virologist, in interpretation and manuscript preparation is greatly acknowledged.

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