Studies on the Major Common Precipitating Antigen of Capripoxvirus

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SUMMARY

The proteins of sheep pox, goat pox, sheep and goat pox and lumpy skin disease (Neethling) viruses were labelled with $^{35}$S-methionine. The major structural polypeptides of these viruses co-migrated on polyacrylamide gels, demonstrating the very close biochemical relationship between them. Using the agar gel immunodiffusion (AGID) test with radiolabelled antigen preparations, a major common precipitating antigen was identified. This co-migrated on polyacrylamide gels with one of the major structural polypeptides [mol. wt. 67000 (67K)]. The use of $^{35}$S-methionine-labelled antigen preparations considerably improved the sensitivity of the AGID test as a diagnostic test for capripoxvirus antibody detection.

INTRODUCTION

The agar gel immunodiffusion test (AGID) has been used to demonstrate the serological relationship between members of the Orthopoxvirus group (Esposito et al., 1977), the Avipoxvirus group (Uppal & Nilakantan, 1970) and the Capripoxvirus group (Sharma & Dhanda, 1971a; Pandey & Singh, 1972). Sheep and goats that have recovered from capripox infection develop precipitating antibodies that can be detected between 10 and 50 days post-infection (Lefevre, 1983). The results of investigations into the number of antigens of the capripoxvirus group have been confusing (for review, see Kitching, 1983), probably due to the different methods used by different workers for the preparation of antigens. As few strains of capripoxvirus will grow on the chorioallantoic membrane of the developing chick embryo, antigens for the AGID test have been prepared from infected animal tissue (Bhambani & Krishna Murty, 1963; Sharma & Dhanda, 1971a; Subba Rao & Malik, 1983). There is one report of the production of capripox precipitating antigen from tissue culture (Al-Bana, 1978). The difficulty in producing standard and reproducible antigens added to the different methods used to produce antibody has delayed the acceptance of the AGID test as a useful serological test for capripox.

The present work describes the production of a $^{35}$S-methionine-labelled capripoxvirus antigen and its use in the AGID test and in the study of the major common precipitating antigen.

METHODS

Materials. Trypsin, trypsin inhibitor and DNase were obtained from Sigma. $^{35}$S-Methionine, SJ.204 (1400 Ci/mmol), and $^{14}$C-methylated protein mixture, CFA.626, were obtained from Amersham.

Viruses. Isolates of Nigeria sheep pox, Yemen goat pox, Kenya sheep and goat pox, Oman sheep pox, Sudan sheep pox and Sudan goat pox, as previously described, were used (Kitching & Taylor, 1985a; Kitching & Mellor, 1985). A vaccine strain of lumpy skin disease (LSD) Neethling virus was supplied by Dr B. Erasmus of the Veterinary Research Institute, Onderstepoort, Republic of South Africa.

Sera. Sera were collected from sheep and goats which had recovered from infection with the isolates of viruses listed above. Sera against LSD were supplied by Dr B. Erasmus, and sera against contagious pustular dermatitis...
Polyacrylamide gel electrophoresis (PAGE). Purified virus polypeptides and the proteins that precipitated in the agar gel taken from Petri dishes were analysed using discontinuous PAGE (Laemmli, 1970). Samples of purified virus and of agar containing radiolabelled precipitated viral proteins were heated for 3 min in a water-bath held at 100 °C, with 0·2 vol. of a fivefold concentrated dissociating buffer (Laemmli, 1970). The proteins were separated in a vertical 20 cm gel. The stacking gel contained 4% acrylamide in 0·125 M-Tris-HCl pH 6·8, 0·1% SDS, and the separating gel contained 10% acrylamide in 0·375 M-Tris–HCl pH 8·8, 0·1% SDS. The running buffer was 0·025 M-Tris, 0·192 M-glycine, 0·1% SDS. Electrophoresis was carried out at a constant voltage of 60 V for 16 h, by which time the bromophenol blue marker dye had reached the bottom of the gel. The gel was fixed for a minimum of 30 min in 10% glacial acetic acid with 20% methanol, and prepared for fluorography according to the method of Bonner & Laskey (1974).
RESULTS

The main structural polypeptides of the capripoxvirus isolates used in this study appeared to be identical when analysed by PAGE. More than 20 co-migrating polypeptide bands could be distinguished, five of which were strongly labelled relative to the other polypeptides (Fig. 1). The molecular weight of these five major polypeptides were 67000 (67K), 64K, 47K, 30K and 22K, as determined by comparison with $^{14}$C-labelled polypeptide markers.

The labelled Nigeria isolate antigen prepared from tissue culture produced one major and one or two additional precipitation lines in the AGID test with convalescent sera from animals infected with Nigeria sheep pox, as detected with Coomassie Brilliant Blue stain. The development and appearance of the precipitation lines were similar to that seen using antigen prepared from a papule collected from an infected animal (Fig. 2). The tissue culture-prepared antigen was thus suitable for use in diagnostic and comparative tests.

The number of precipitation lines formed in agar gel was dependent on the time interval
between infection and the collection of sera and also on the severity of clinical signs shown by the donor animals. The major line developed with sera collected 15 days after infection and a second line could be observed when sera collected at 37 days were used (Fig. 3a). A third line was observed only with sera collected 53 days post-infection from sheep that had recovered from a severe infection; it could no longer be detected by day 85 (results not shown). However, the first and second lines were still observed using sera collected on day 113. No precipitation lines were observed using antigen prepared from uninfected tissue culture.

If the gels were subsequently prepared for autoradiography, the sensitivity of the AGID test was markedly improved. Thus, the second precipitation line, not seen in sera until day 42 post-infection using Coomassie Brilliant Blue stain, was detected with day 21 post-infection sera using autoradiography (Fig. 3b). Sera from sheep or goats recovered from mild capripox infection or following vaccination also frequently failed to produce a precipitation line detectable with Coomassie Brilliant Blue, whereas a line was readily demonstrable using autoradiography. Non-specific visible precipitation lines, in many instances due to reaction between antibodies to bovine serum, in the test serum, and bovine serum present in the antigen, were not detected using radiolabelled antigen followed by autoradiography.

The labelled Nigeria antigen was tested against sera collected at intervals from sheep which were infected and had recovered from infection with the Sudan sheep or goat isolates. Two precipitation lines were observed in the same sequence and time intervals as had been observed with the convalescent sera from sheep recovered from infection with the Nigeria isolate (Fig. 4, reaction with Sudan goat pox antisera shown). Labelled antigen prepared from the Nigeria, Yemen, Kenya and LSD virus isolates were tested in various combinations with sera collected from sheep that had recovered from infection with the Nigeria, Yemen, Kenya, Sudan sheep or Oman virus isolates, and with cattle sera against LSD virus (Fig. 5). The precipitation lines between heterologous and homologous reactants joined without the formation of spurs, irrespective of which of the four antigens was used in the central well, thus demonstrating the presence of a common antigen. The development of one or two precipitation lines was not dependent on the sera and antigen being homologous, but on the relative concentrations of the reactants. When the labelled Nigeria antigen was used with sera collected from a lamb that had recovered from severe CPD infection, a single precipitation line was observed from day 22 after the development of clinical signs (Fig. 6). This formed a line of identity with the major precipitation line of the control capripox-positive sera in the adjacent wells. However, no radiolabelled precipitation line was observed with sera from lambs that had experienced milder CPD infection.

Analysis by PAGE of the major radiolabelled precipitating antigen using different combinations of antigen and antisera showed that in each instance this major precipitating antigen co-migrated (Fig. 7). The molecular weight of this antigen was 67K, as determined by comparison with $^{14}$C-labelled polypeptide markers. This antigen co-migrated with the 67K structural polypeptide present in the purified capripoxvirus isolates (Fig. 8).

**DISCUSSION**

Sera from sheep, goats and cattle that had recovered from infection with capripoxvirus produced a major common precipitation line on AGID. This line was the first and frequently the only line to develop. With those sera which produced more than one precipitation line, the number of precipitation lines observed using homologous or heterologous antigen was the same.

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Fig. 2. Coomassie Brilliant Blue-stained gels comparing tissue culture and nodular antigens. (a) Tissue culture-derived radiolabelled Nigeria antigen (central well). Wells 1, 3 and 5, negative serum; wells 2, 4 and 6, positive Nigeria antisera. (b) Antigen derived from a skin papule of a sheep isolate (central well). Wells 1 to 6 as in (a).

Fig. 3. (a) Coomassie Brilliant Blue-stained gel, showing development of precipitation lines, using radiolabelled Nigeria antigen (central wells). Wells 1 to 12, sera from sheep inoculated with Nigeria sheep isolate days 0, 7, 15, 21, 29, 37, 42, 53, 58, 70, 85 and 113 post-inoculation respectively. (b) Autoradiography of agar gel shown in (a).
This result indicated that the number of lines produced by immune sera and antigen was not necessarily related to their homogeneity, but to the relative concentrations of the reactants. The use of the $[^{35}\text{S}]$methionine-labelled antigen has improved the sensitivity of the AGID and increased the duration of time, to over 1 year after infection, in which precipitating antibodies can be detected. The use of labelled antigens of isolates of sheep pox virus, goat pox virus, sheep and goat pox virus and LSD virus, produced under similar conditions, has also made possible a direct comparison between homologous and heterologous precipitating reactants. The major $[^{35}\text{S}]$methionine-labelled precipitating antigen of the four isolates of capripoxvirus used in this study formed a common line of identity when used in adjacent wells in the AGID test. They also co-migrated when analysed by PAGE. These similarities suggested that this major common antigen was the same polypeptide in all four isolates, and was probably shared by all isolates of capripoxvirus.

It was found that the AGID test could not be used to distinguish between the different members of the Capripoxvirus group. Reports by other research workers (for review, see Lefevre, 1983) that goat pox antigen and sheep pox antigen produced more precipitation lines with homologous sera than with heterologous sera could not be confirmed using tissue culture-derived antigen. Esposito et al. (1977) examined the serological relatedness of monkey pox, variola and vaccinia viruses. By adsorbing antisera to one of these orthopoxviruses with a heterologous antigen, they were able to remove common antibodies and leave specific homologous antibodies which could be demonstrated using the AGID test. Al-Bana (1978), using the same technique, was able to distinguish between a Turkish strain of sheep pox and a Turkish strain of goat pox, using hyperimmune but not convalescent sera.

The cross-reaction between capripox antigen and sera from a lamb recovering from severe CPD infection showed that CPD virus shares a precipitating polypeptide of the same molecular weight as capripoxvirus. This cross-reaction had previously been reported by Sharma & Dhanda (1971b) and Subba Rao et al. (1984), but considered of little importance by those using the AGID test for the diagnosis of capripox (Dardiri, 1978). However, it is our view that because of this cross-reaction with CPD serum the AGID test cannot be used on an individual basis. Nevertheless, it could be of considerable use in epidemiological surveys when large numbers of sera are being examined, and in assessing response to capripox vaccination, where pre- and post-vaccination sera are available. Experiments (R. P. Kitching, unpublished) have shown that immunity to challenge with virulent capripoxvirus persists in vaccinated animals when precipitating antibodies can no longer be detected with the $[^{35}\text{S}]$methionine-labelled antigen. This indicated that the absence of detectable precipitating antibodies cannot necessarily be equated with susceptibility to capripox infection.

The main structural polypeptides of the capripoxvirus isolates used in this study appeared to be identical when analysed by PAGE. In particular, they all contained two polypeptides of mol. wt. 67K and 64K, the larger of which co-migrated on PAGE with the major common precipitating antigen. Preliminary work using detergent-treated purified virus preparations showed that this antigen was located on the outer membrane of the virus particle (results not shown).

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Fig. 4. Autoradiography of agar gel, showing development of precipitation lines using antisera to Sudan goat pox. Radiolabelled Nigeria antigen (central wells). Wells 1 to 12, sera from sheep inoculated with Sudan goat isolate days 0, 10, 18, 35, 38, 53, 82, 125, 166, 194, 216 and 264 post-inoculation respectively.

Fig. 5. Autoradiograph of agar gel, showing serological relatedness between capripoxvirus antigens. Radiolabelled Nigeria (N), Yemen (Y), LSD (L) and Kenya (K) antigens with antisera against Nigeria sheep (N), Yemen goat (Y), Oman sheep (O), LSD (L), Kenya sheep and goat (K) and Sudan sheep (S) isolates.

Fig. 6. Autoradiograph of agar gel, showing cross-reaction between capripoxvirus antigen and antisera to CPD. Central wells, radiolabelled Nigeria antigen; wells +, positive control Nigeria antisera; wells 1 to 8, sera from sheep infected with CPD days 1, 8, 15, 22, 29, 36, 43 and 50 respectively, after onset of clinical signs.
Fig. 7. Autoradiograph of PAGE analysis of the major radiolabelled agar gel precipitation line. Lane 1, Nigeria antigen with Nigeria sheep isolate antiserum from sheep; lane 2, Nigeria antigen with Yemen goat isolate antiserum from sheep; lane 3, Nigeria antigen with LSD antiserum; lane 4, Nigeria antigen with Kenya sheep and goat isolate antiserum from sheep; lane 5, Nigeria antigen with CPD antiserum from sheep; lane 6, Nigeria antigen with Nigeria sheep isolate antiserum from goat; lane 7, Yemen antigen with Yemen goat isolate antiserum from sheep; lane 8, Kenya antigen with Kenya sheep and goat isolate antiserum from sheep; lane 9, LSD antigen with LSD antiserum; lane 10, LSD antigen with Yemen goat isolate antiserum from sheep.

The results reported here have demonstrated the close biochemical relationship between members of the capripox group of viruses. Previous work (Davies & Otema, 1981; Kitching & Taylor, 1985a, b; Kitching & Mellor, 1985) has shown the close antigenic and epidemiological relationship between sheep pox virus, goat pox virus, sheep and goat pox virus and LSD virus. Taken together, these results would cast doubt on the justification for the continued classification of the capripoxviruses into distinct types (Matthews, 1982).
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REFERENCES


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