Identification of Caprine Arthritis–Encephalitis Retrovirus Proteins in Immunodiffusion Precipitin Lines

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SUMMARY

Precipitin lines formed between serum from a goat infected with caprine arthritis–encephalitis virus (CAEV) and radiolabelled viral proteins in polyethylene glycol-concentrated culture medium were excised from immunodiffusion (ID) plates and analysed by polyacrylamide gel electrophoresis. The two major precipitin lines contained the 135000 mol. wt. glycoprotein (gp135) and the internal 28000 mol. wt. structural protein (p28). This method obviates the use of purified proteins or monospecific antisera to positively determine viral constituents in ID precipitin lines formed between a crude antigen preparation and antiserum against whole virus.

The non-oncogenic retrovirus of goats, caprine arthritis–encephalitis virus (CAEV), causes severe chronic arthritis and a variety of other pathological processes in dairy goats (Crawford et al., 1980a, b). Serology, pathology and clinical reports indicate that the infection is widespread among goats in North America, Europe and Australia but relatively uncommon in South America and Africa (Adams et al., 1984).

The serology reported from this laboratory has utilized immunodiffusion (ID) with a reference line resulting from precipitation between serum from a CAEV-infected goat and the internal 28000 mol. wt. structural protein (p28) (Crawford & Adams, 1981; Roberson et al., 1982; Adams et al., 1983a, b, 1984). Others have used polyethylene glycol (PEG)-concentrated medium from ovine progressive pneumonia virus (OPPV)- or maedi/visna virus (MVV)-infected cultures in ID and purified MVV for the enzyme-linked immunosorbent assay (ELISA) (D. Houwers, personal communication). In three comparisons, a higher number of seropositive goats was identified using PEG-concentrated antigen in ID than with the ID which detects antibody to p28 (J. Pearson & D. S. Adams, unpublished observations; M. Dawson & D.S. Adams, unpublished observations; D. S. Adams et al., unpublished observations, respectively).

Recently, Johnson et al. (1983) found that goats experimentally infected with CAEV had much higher titres of serum and synovial antibody directed against virion surface glycoproteins than against p28. Therefore, it was suspected that assays with the PEG-concentrated antigen involved one or more of these viral surface glycoproteins. Because we had found that more infected goats could be identified using the PEG-concentrated antigen than with the assay that detected p28 antibody, it was decided to determine what CAEV proteins were immunoprecipitated in ID. Here, we describe a novel method of positively identifying the proteins of CAEV that are found in the two major immunoprecipitin lines formed between PEG-concentrated cell culture medium and antiserum from a CAEV-infected goat.

Caprine synovial membrane cultures were infected with a CAEV isolate (75G63) (Crawford et al., 1980a) which had been cloned three times. Cultures were maintained in Dulbecco's minimal essential medium (DMEM) with 5% foetal calf serum and antibiotics as previously described (Klevjer-Anderson & Cheevers, 1981). Culture medium was concentrated 100-fold...
with PEG (Cutlip et al., 1977), and ID was performed as previously described (Adams et al., 1984). The reference serum used as a positive control was obtained from a goat (78G77) which had been experimentally infected with CAEV 5 years previously. This serum formed two distinct lines in ID when the concentrated medium was used as antigen: a wide line at dilutions of 1:4 or less (designated line A) and a narrow line at dilutions from 1:16 to 1:256 (designated line B). Line A fused with the reference line obtained with 78G77 serum and the ether-extracted CAEV antigen used in previous studies (Adams et al., 1984, 1983a, b; Crawford & Adams, 1981).

Viral proteins were radiolabelled to determine the proteins present in each precipitin line. Four h after addition of methionine-deficient DMEM, [35S]methionine (0.01 mCi/ml) and [3H]glucosamine (0.01 mCi/ml) were added to the cultures. After 4 days the medium was collected and concentrated 100-fold with 7500 or 20000 mol. wt. PEG. Serum from goat 78G77 was diluted in phosphate-buffered saline (PBS). Line A, formed by undiluted serum, and line B, formed by serum diluted 1:64, were excised from the agar and washed for 48 h with four changes of PBS to remove unprecipitated proteins and free isotope. The excised agar was melted by boiling and dried under vacuum to decrease the volume. The dried material was then boiled in SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer [0.062 M-Tris-HCl pH 6-8, 0.2% (w/v) SDS, 5% 2-mercaptoethanol, 7.5% glycerol] for 3 min and applied to a 3.5% polyacrylamide stacking gel with a discontinuous 7.5 to 17.5% polyacrylamide resolving gel (Johnson et al., 1983).

PEG-concentrated culture medium labelled with [35S]methionine and [3H]glucosamine was also analysed by immunoprecipitation with control and immune goat sera, followed by SDS-PAGE of the precipitated proteins. In some experiments, cell debris and free virions were removed by differential centrifugation prior to PEG concentration. An initial clarification (SW41 rotor, 20000 r.p.m., 30 min) was followed by centrifugation of the supernatant (SW27 rotor, 24000 r.p.m., 100 min) to remove virions. The sera used in these immunoprecipitations, in addition to 78G77 described above, were 82G15 (CAEV-free control), 80G104 (immunized with line B) and 82G95 (naturally affected with CAE but negative for serum anti-p28 antibody by ID).

Concentrated supernatants were adjusted to a volume of 0.9 ml in TEN buffer (0.02 mm-Tris-HCl pH 7.4, 1.0 mm-EDTA, 100 mm-NaCl) and NP40 was added to a final concentration of 1.0%. After sonicating the mixture for 15 s at 40 W, SDS was added to a final concentration of 0.1%. The mixture was centrifuged at 100000 g for 1 h and the supernatant was re-sonicated. Immunoprecipitations were carried out according to the method of Shapiro & August (1976). Six-hundred thousand c.p.m. (trichloroacetic acid-precipitable) originating from infected or control cultures were incubated with 0.01 ml antiserum overnight at 4 °C. Then, 0.1 ml of a 10% suspension of staphylococci with surface Protein A (Pansorbin, Calbiochem-Behringer) was added, and the mixture was incubated at 4 °C for 1 h. The resulting pellet was washed once with TEN–2 m-NaCl, twice with TEN in 0.1% NP40, twice with TEN, and finally once with TEN–2 m-NaCl. The pellet was resuspended in SDS-PAGE sample buffer, boiled for 3 min, and centrifuged at 3000 g for 20 min. The supernatant was applied to a 7.5 to 17.5% polyacrylamide gradient slab gel. Molecular weight standards were [14C]methylated proteins composed of myosin 200000, phosphorylase b 92500, bovine serum albumin 69000, ovalbumin 46000, carbonic anhydrase 30000, and lysozyme 14300 mol. wt. After electrophoresis the gel was impregnated with En3Hance (New England Nuclear), dried under vacuum and exposed to X-ray film at -70 °C.

Definitive identification of the viral proteins in the precipitin lines A and B can be seen in Fig. 1. Line A (lane 1), formed at low dilution of 78G77 serum, was due to the precipitation of the major internal structural protein of CAEV, p28. Line B (lane 2), formed at high dilution of 78G77 serum, is clearly due to precipitation of the 135000 mol. wt. glycoprotein (gp135) of CAEV. Furthermore, serum from the goat that was immunized with line B immunoprecipitated with gp135 (lane 5). Additional virion antigens (Johnson et al., 1983; R. P. Gogolewski et al., unpublished results) were precipitated from concentrated culture medium by 82G95 serum (lane 3) and 78G77 serum (lane 4).
An additional protein of mol. wt. approximately 250000 precipitated in the presence of all three sera. This protein is not immunoprecipitated when purified virus is used as antigen (R. P. Gogolewski et al., unpublished results). We therefore performed immunoprecipitation reactions using concentrated medium of uninfected cultures and of CAEV-infected cultures from which the virions had been removed. This 250000 mol. wt. protein, one at 70000 mol. wt. and several minor bands were precipitated from concentrated medium of infected or uninfected cells in the presence of Protein A-containing staphylococci alone (Fig. 2, lanes 5 and 10). Serum from goat 78G77 predominantly immunoprecipitated gp135 and p28 from virus-free medium of infected cultures (lane 1). These proteins were not precipitated by serum from the control goat (82G15) (lane 4), and the 78G77 serum did not identify these proteins in uninfected culture medium (lane 6). Serum from 82G95 and 80G104 predominantly immunoprecipitated the gp135 from infected (lanes 2 and 3), but not from uninfected (lanes 7 and 8) cell culture medium.

These data demonstrate that PEG-concentrated medium from CAEV-infected cultures can be used in ID and that the two lines formed by the reference serum, 78G77, are due to precipitation of the viral proteins p28 and gp135. Both of these proteins were the major viral constituents immunoprecipitated after removal of virions and therefore are probably exported

Fig. 1. PAGE analysis of PEG-concentrated CAEV antigen and immune goat serum. Lane 1, ID precipitin line formed with undiluted 78G77 serum; lane 2, ID precipitin line formed with 78G77 serum diluted 1:64; lane 3, immunoprecipitation by serum from a goat (82G95) with naturally acquired CAE (negative in ID to p28 but positive to gp135); lane 4, immunoprecipitation by 78G77 serum (positive in ID to p28 and gp135); lane 5, immunoprecipitation by 80G104 (immunized with gp135 ID precipitin lines). Mol. wt. standards are shown x 10^-3.
Fig. 2. PAGE analysis of immunoprecipitates of PEG-concentrated medium (without virions) from CAEV-infected (lanes 1 to 5) and uninfected (lanes 6 to 10) cell cultures. Lanes 1 and 6, 78G77 goat serum; lanes 2 and 7, 82G95 goat serum; lanes 3 and 8, 80G104 goat serum; lanes 4 and 9, 82G15 (CAEV-free) serum; lanes 5 and 10, Protein A-containing staphylococci without serum.

to the medium from infected cells or perhaps released from virions after budding. It should be noted that the apparent mol. wt. of 135000 obtained here for the major surface glycoprotein of CAEV is at slight variance with our previous estimate of 125000 (Johnson et al., 1983). This difference may have been due to the use of a longer slab gel in the present study, but measurements of mol. wt. are inherently less precise in this region of the gel which also may have contributed to the observed difference.

The finding of a 70000 mol. wt. protein and several minor bands in concentrated medium from control cells indicates that they are products of host cell genes. If they are the same ones seen in gels from immunoprecipitations of purified virus (Johnson et al., 1983, R. P. Gogolewski et al., unpublished results) then they must co-purify with or be incorporated into virions. Furthermore, since these bands appeared in lanes where no antiserum had been added, they either bound the staphylococci alone or precipitated spontaneously.

The technique of using radiolabelled viral proteins in ID and analysing excised immunoprecipitin lines by PAGE circumvented the need to use purified CAEV proteins or monospecific antisera to determine the identity of the immunoprecipitin lines formed between serum from infected goats and PEG-concentrated medium from infected cultures. Now that the lines have been characterized we hope to determine which protein, the p28 or the gp135, is the most appropriate antigen for serology. This simple technique offers an attractive alternative to protein purification in other systems where such identification is necessary.

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