Simple and Rapid Method for Production of Whole-Virus Antigen for Serodiagnosis of Caprine Arthritis-Encephalitis Virus by Enzyme-Linked Immunosorbent Assay

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Polyethylene glycol (PEG) was used to produce whole-virus antigen derived from tissue culture cells infected with a Canadian strain of caprine arthritis-encephalitis virus. PEG antigen batches were obtained after precipitation and concentration of infected tissue culture material with PEG 8000 and final treatment with sodium dodecyl sulfate. The optimum time of harvest of tissue culture extracted material to produce the maximum amount of viral proteins was determined in roller bottles, after cocultivation of infected and noninfected fetal lamb corneal cells. Samples from day 9 to day 25 postculture were collected and processed. By Western blotting, the optimum time of harvest was found to be day 25 following the coculture. Two large batches of PEG antigen were prepared at the optimum time of harvest. Both batches gave similar results when tested by Western blotting and enzyme-linked immunosorbent assay (ELISA), using reference control sera from infected and noninfected goats. For further testing in ELISA, cutoff values and ratios were determined for PEG batch 1, using 200 known serum samples from goats free of the disease. The PEG antigen batch was compared with an in-house ELISA antigen in a kinetic mode, using 498 serum samples from field goats. The in-house ELISA antigen was produced following two rounds of ultracentrifugation and treatment with sodium dodecyl sulfate (R. A. Heckert, W. B. McNab, S. M. Richardson, and M. R. Briscoe, Can. J. Vet. Res. 56:237–241, 1992). The PEG antigen batch was found suitable for ELISA, with a relative specificity of 100% and a relative sensitivity of 99.4% compared to the in-house ELISA antigen. This method of antigen production for ELISA was found to be rapid, inexpensive, and reliable for the diagnosis of caprine arthritis encephalitis, without requiring the use of sophisticated laboratory equipment.

Caprine arthritis-encephalitis virus (CAEV) belongs to the Retroviridae family and the subfamily Lentivirinae. The virus is distributed worldwide and causes leuko-encephalomyelitis in goat kids (13), chronic progressive arthritis-synovitis, indurative mastitis, and chronic interstitial pneumonia in adult goats (6, 9, 12). The development of clinical disease takes a few months to a few years, with infections in most animals remaining subclinical (7, 15). For both the clinical and asymptomatic forms, the CAEV causes a lifetime infection despite humoral and cellular immune responses (8), with no known effective treatment; the infected animals remain reservoirs of the virus for their entire lives. Transmission between infected and susceptible animals occurs mainly through colostrum and milk consumption (1, 15). Contact transmission between goats of all ages has also been demonstrated (15). The early detection of the infected animals and their segregation and/or eradication from the flock form an efficient practice to limit the spread of the virus (17). The infection is primarily detected by the demonstration of specific CAEV antibodies in the body fluids of the infected goats (15). The agar gel immunodiffusion (AGID) assay and the enzyme-linked immunosorbent assay (ELISA) are routine serological test methods used for the serological detection of CAEV antibodies (2, 10, 11, 14, 20, 25). Antigens used for these laboratory methods are commonly extracted and purified from tissue culture (TC) infected cell material, using complex manipulation and/or expensive equipment for the purification steps (2, 4, 11, 20, 25). Recombinant technology is also used to produce recombinant viral proteins (5, 16, 18). Even though this modern approach is usually excellent for producing large amounts of recombinant viral proteins that are useful in ELISA, their production and purification require sophisticated approaches that are not always available to a diagnostic laboratory. This paper describes a very simple, rapid, and reliable method for the optimal production of TC-derived antigens suitable for ELISA for the diagnosis of CAEV.

MATERIALS AND METHODS

Virus isolate. A pure Saanen adult goat from New Brunswick, Canada, with a CAEV-positive serological status (determined by AGID) and a story of severe arthritis in all legs, swollen knees, and lameness was euthanized after cesarian delivery of triplets. Synovial fluid and various tissues (synovial membranes, lungs, kidney, and mammary gland) were aseptically collected for TC isolation and were also fixed in formaldehyde for confirmation of CAE disease by histopathology. A virus producing giant cells and syncytia with a slow-evolving cytotoxic effect was isolated from the knees, mammary gland, and lungs, at the third or fourth passages following cocultivation of explant tissues with fetal lamb corneal (FLCor) or fetal goat synovial membrane (FGSm) culture cells. The viral stock SK167a was derived from the infected FGSm culture cells cocultured with lung explants. The infected cells were found to be free of bovine diarrhea virus, parainfluenza-3 virus, and mycoplasma, as determined by indirect immunofluorescence (IFA) and a Mycoplasma detection kit (Canadian Life Technol-
of viral antigen for ELISA. For this purpose, more roller bottles were grown and harvested all together, at the predetermined OTH.

Precipitation and concentration of viral proteins with PEG. For each roller bottle collected at the indicated time intervals and for a lot of roller bottles harvested at the predetermined OTH, the viral proteins were extracted, precipitated, and concentrated using PEG 8000 (Fisher Scientific Ltd., Nepean, Ontario, Canada). Briefly, frozen roller bottles (cells and medium) were freeze-thawed consecutively three to four times. The collected material was transferred to 50-ml centrifugation tubes and centrifuged at 2,000 rpm for 20 min to clarify the supernatant. The supernatant was collected and measured prior to be transferred to 250-ml centrifuge bottles. It was mixed with 30% PEG 8000 in 0.4 M NaCl at the ratio of 2:1 (supernatant-PEG). The mixtures were incubated overnight at 4°C with constant gentle agitation or rocking. The precipitated proteins were recovered the following day by centrifugation at 2,000 rpm for 20 min. The supernatant obtained after PEG 8000 precipitation was discarded, and the pellets were dried by inverting the tubes on absorbent paper towels for 5 to 10 min. The pellets were dissolved in phosphate-buffered saline (PBS) containing 0.15 M NaCl, using 1/100 of the starting supernatant volume, in which the protease inhibitor phenylmethylsulfonyl fluoride (Canadian Life Technologies Inc., Burlington, Ontario, Canada) (11) was added. The virus antigens were collected at the indicated time intervals and for a lot of roller bottles harvested at the predetermined OTH.

RESULTS

Time course study of harvest. For the time course study, dissolved proteins of the supernatant and pellet fractions obtained following precipitation, dialysis, and concentration with PEG 8000 were tested by WB at some time points for comparison. A positive goat serum recognizing the capsid antigen of CAEV (gag p28) was used. Samples were from TCS day 19 of the negative control roller bottle and TCS days 19, 23, and 25 of infected roller bottles. WB showed no reactivity of a mix of pellet and supernatant material for the negative control (Fig. 1, lane 6). In contrast, a strong immune reaction was
CAEV-positive goat serum was used. Specific bands were detected at 8, 21 (lane 9), 23 (lane 10), and 25 (lane 11). A reference polyclonal antibody against CAEV for all tested infected TCS, as compared to their corresponding supernatant fractions (Fig. 1, compare lanes 2 and 3 with lanes 4 and 5). Interestingly, dissolved pellet of TCS day 25 (Fig. 1, lane 1) showed stronger reaction than dissolved pellet of TCS day 19 (Fig. 1, lane 3), although there were fewer infected cells in the system due to higher cytolysis at that time. To determine the OTH of the pellet fraction, the infected dissolved pellet fractions at all the time points were tested in WB following a similar approach. Figure 2 presents the results for the infected pellet fractions of TCS days 9, 11, 13, 15, 17, 19, 21, 23, and 25 and TCS day 17 of the negative control pellet. No signal was found in the pellet fraction of the noninfected cells at TCS day 17 (Fig. 2, lane 2). Weak specific CAEV bands were seen in WB on TCS day 9 (Fig. 2, lane 3). The intensity of the bands increased gradually from this time point until TCS day 25, where the strongest signal was observed (Fig. 2, lane 11). The CAEV polyclonal goat serum detected three specific bands, at approximately 10, 25, and 42 kDa. The protein of ~25 kDa was the most prominent band (Fig. 2).

Antigen batches for ELISA. From the above results, two large antigen-derived PEG batches from eight (batch 1) and six (batch 2) roller bottles of virus-infected and corresponding noninfected FLCor cells were produced. The roller bottles were frozen at TCS days 23 to 25 for PEG batches 1 and 2. An identical approach using PEG 8000 to precipitate and concentrate the viral proteins was followed. To ensure the PEG batches were comparable to the smaller scale antigens produced at TCS days 23 to 25 from the previous experiment, WB were set up. The pellet fraction of the PEG batch 1 revealed 3 bands of similar molecular weight found with smaller batches at TCS days 23 to 25 (compare Fig. 2 and 3). However, 2 minor bands between 25 and 42 kDa, which were not detected previously, were found. The band at ~25 kDa was still the most abundant antigenic viral protein in this viral stock. Again, weaker signals were detected with the supernatant fraction (Fig. 3, lane 2). PEG batch 2 gave similar results (data not shown).

i-ELISA. Both PEG batches of viral antigens were used in an i-ELISA following a conventional procedure (11, 22). They were tested at different dilutions against known positive and negative reference sera from CAEV-infected and noninfected goats respectively, with a confirmatory assay in a static mode. Table 1 indicates the OD414 readings obtained for the positive reference serum tested against a positive antigen batch and its corresponding negative antigen control. Both PEG batches were found suitable in ELISA, giving good discrimination between the positive and the negative antigen wells, and were found comparable to each other (Table 1). For further in-depth comparison and validation, PEG batch 1 was tested with 498 serum samples from field goats in a kinetic mode, after the

FIG. 1. WB of supernatant and dissolved pellet fractions of PEG-derived CAEV whole-virus antigens at different TCS days, following precipitation, dialysis, and concentration with PEG 8000. Lanes 1 to 3, PEG-derived antigen fractions from the infected dissolved pellet of TCS days 25 (lane 1), 23 (lane 2), and 19 (lane 3); lanes 4 to 5, PEG-derived antigen fractions from the infected supernatant of TCS days 23 (lane 4) and 19 (lane 5); lane 6, PEG-derived fraction from TCS day 19 of noninfected cells. A reference CAEV-positive goat serum recognizing the capsid antigen of the virus was used. A specific band of around 25 kDa was detected in lanes 1 to 5.

FIG. 2. WB of the dissolved pellet fractions of PEG-derived CAEV whole virus antigens at increasing TCS days and following precipitation, dialysis, and concentration with PEG 8000. Lane 2, PEG-derived fraction from TCS day 17 of noninfected dissolved pellet; lanes 3 to 11, PEG-derived antigen fractions from infected dissolved pellet at TCS days 9 (lane 3), 11 (lane 4), 13 (lane 5), 15 (lane 6), 17 (lane 7), 19 (lane 8), 21 (lane 9), 23 (lane 10), and 25 (lane 11). A reference polyclonal CAEV-positive goat serum was used. Specific bands were detected at around 10, 25, and 42 kDa (refer to molecular weight [MW] markers [in thousands], lane 1).

FIG. 3. WB of supernatant and dissolved pellet fractions of PEG-derived CAEV whole-virus antigen batch 1 following precipitation, dialysis, and concentration with PEG 8000. Lanes 1 and 2, dissolved pellet (lane 1) and supernatant (lane 2) fractions of PEG-derived antigens from eight roller bottles at TCS day 23 of infected cells; lane 3, dissolved pellet fraction of noninfected cells. A reference polyclonal CAEV-positive goat serum was used. Specific bands were detected at approximately 10, 25, and 42 kDa. Also, two other bands were detected between 25 and 42 kDa.


**DISCUSSION**

This work describes a very simple and rapid method of antigen production suitable in i-ELISA for the serodiagnosis of CAEV. Whole virus antigen obtained from TC was simply pelleted with PEG, dialyzed against water and PBS, and concentrated with PEG again, prior to being treated with SDS. The optimum time of antigen production was determined by a time course study, following cocultivation of CAEV-infected and noninfected cells. The OTH, using the CAEV Canadian isolate (SK167a), was determined to be at TCS days 23 to 25. Several strains of CAEV exist, which differ in virulence and antigenicity (15) and which do not have the same behavior in a culture system in vitro (3). It is recommended that each laboratory determine the best time to harvest the viral stock that will be used to produce the antigen batch, prior to initiating the production of a larger batch of antigen production suitable in ELISA. It is possible that a slow cytopathic strain, as used in this study, will perform better than a highly cytopathic strain which induces a faster destruction of TC cells.

The production of the PEG antigen did not require the use of expensive equipment such as an ultracentrifuge or complex manipulations involved in pressure filtration, chromatography, and/or gradient centrifugation required for the production of other reported TC-derived CAEV antigen batches suitable for ELISA (2, 11, 20). In fact, the PEG antigen was found to be comparable to our in-house antigen used previously at our laboratory for the diagnosis of CAEV (11). Moreover, the PEG antigen batches were found stable at −70°C for many months, in suspension or when the ELISA plates were coated with them. The benefits of the PEG antigen were its rapidity, simplicity of production, reproducibility, and lower cost of production. A single batch of eight infected roller bottles provided a capacity of screening of around 24,000 serum samples.

We were the first to report the use of SDS for the final treatment of antigens used in ELISA for Maedi-visna virus, a closely related retrovirus (21). This ionic detergent was also used to produce our in-house CAEV ELISA antigen (11), as well as our CAEV PEG antigen. The same final treatment has been used also by others and found to be the best approach in the treatment of CAEV whole-virus antigens from TC, although they were obtained using different purification approaches (4, 23). SDS at a low concentration seems mainly to expose linearized antigenic CAEV or MV epitopes recognized by specific antibodies. Using WB and a polyclonal serum from an infected goat, at least three major immunogenic viral proteins in the PEG antigen were detected, at approximately 10, 25, and 42 kDa, representing most probably the reported trans-membrane envelope protein (40 kDa), the capsid protein (28 kDa), and another minor (p15) viral protein (24). Two other minor protein bands between 25 and 42 kDa were also detected, which may represent degradation subproducts of higher-molecular-weight proteins. The membrane envelope gp 130 (24) was not demonstrated in this system. This might be because of the lack of the corresponding specific antibody in the reference polyclonal goat serum used in WB, or most probably because of degradation or loss of conformational epitopes during the antigen production and/or WB procedures. ELISAs based on whole-virus antigens have been shown to be effective for the diagnosis of CAEV-infected animals (2, 11, 20, 25). Recombinant ELISAs have also been developed successfully with recombinant proteins derived from the trans-membrane or capsid genomic sequences regions (5, 16, 18). Although the latter are using single antigenic proteins (5, 16, 18) or a mix of two (16), whole-virus antigens usually comprise a variety of viral proteins for which animals develop antibodies; thus, whole virus antigens might be more sensitive in ELISA than a single protein or the mix of two recombinant proteins.

The i-ELISA can be automated, making this technique useful for the screening of large numbers of sera. The sensitivity and specificity of the i-ELISA depend, however, on the quality of antigens. According to the Office international des épidémiologies, the production of satisfactory antigen preparations has limited routine application of the ELISA for the diagnosis of CAEV (14). Serological methods are required in eradication and control programs for CAEV. For those, many laboratories across the world are still using the AGID test (14), mainly because of the ease and low cost of producing the crude antigen required in the AGID. However, this technique has been shown to be significantly less sensitive than the ELISA, both for CAEV and for Maedi-visna virus (2, 11, 22). Due to the lack of sensitivity of the AGID test method, a more sensitive assay, such as the ELISA, is strongly recommended in eradication campaigns. The use of recombinant protein antigens

**TABLE 2. Comparison between CAEV PEG antigen (batch 1) and CAEV in-house antigen in i-ELISA using a kinetic mode**

| CAEV PEG antigen result | No. with indicated CAEV antigen in-house antigen result: | Total no. |
|-------------------------|----------------------------------------------------------|----------|
|                         | Positive | Negative |          |
| Positive                | 155     | 0        | 155      |
| Negative                | 1       | 340      | 341      |
| Suspicious              | 2       | 0        | 2        |
| Total                   | 158     | 340      | 498      |

*R Relative specificity = number of negative results obtained/number of negative results expected × 100 = 100%. Relative sensitivity = Number of positive and suspicious results obtained/number of positive results expected × 100 = 99.4%.*
and of monoclonal antibodies has been developed to overcome the problems associated with the production and purification of whole-virus antigens suitable for ELISA. Although these newer technologies are reported to be highly sensitive and specific, they are not available to all laboratories involved in the diagnosis of CAEV. The development of a sensitive and specific assay based on whole-virus antigen that could be produced easily with a rapid and cheap methodology, such as the PEG approach, could be very useful for the diagnosis of CAEV.

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