JOURNAL OF VIROLOGY, May 1992, p. 3247–3250
0022-538X/92/053247-04$02.00/0

Vol. 66, No. 5

Transmembrane Protein Oligomers of Caprine Arthritis-Encephalitis Lentivirus Are Immunodominant in Goats with Progressive Arthritis

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Received 12 November 1991/Accepted 12 February 1992

To dissect mechanisms of caprine arthritis-encephalitis lentivirus-induced arthritis, an undefined immunodominant viral glycoprotein, gp90 (G. C. Johnson, A. F. Barbet, P. Klevjer-Anderson, and T. C. McGuire, Infect. Immun. 41:657–665, 1983), was characterized. Monoclonal antibody to gp90 and specific antiserum to *env* gene products demonstrated that gp90 was a transmembrane protein (TM) dimer. Goats with progressive arthritis had high antibody titers to oligomeric and monomeric (38-kDa) TM.

Caprine arthritis-encephalitis lentivirus (CAEV) causes clinical arthritis in 20 to 30% of naturally infected goats (11, 16, 39). Arthritis is usually progressive (5, 13) and is particularly severe in carpal synovial spaces (11). The interpretation that immune responses to CAEV antigens are one cause of arthritis is based on lymphocytes and macrophages in the lesions (1, 12) and on the following results of four experiments. (i) Total immunoglobulin G1 is increased in synovial fluid from arthritic joints (21) with 10 to 100 times more antibody to viral glycoproteins than to other viral proteins (22). (ii) Antibody titers to CAEV surface protein (SU) in synovial fluid predict the severity of arthritis (24). (iii) Goats immunized with inactivated CAEV and challenged with virulent CAEV develop more severe arthritis than CAEV-challenged control goats (27). (iv) Treatment of CAEV-infected goats with antilymphocyte serum and cyclophosphamide prevents lesions without altering virus titers (10).

To determine how immune responses to CAEV cause arthritis, definition of immunodominant viral glycoproteins is necessary. One immunodominant CAEV glycoprotein is SU (22, 24, 25) and another is an undefined glycosylated 90-kDa protein (22). Goats immunized with recombinant vaccinia virus expressing the CAEV *env* gene (rWR-63) make antibody to SU and to 38- and 90-kDa proteins (25). Since the 5′ end of *env* encodes SU (25), it was assumed that the 38- and 90-kDa proteins were from the 3′ end of *env* which encodes the transmembrane protein (TM) of other lentiviruses. The purpose of this paper is to identify immunodominant CAEV gp90 as TM as well as (i) to demonstrate that oligomers with a 38-kDa TM monomer occur and (ii) to show that goats with progressive CAEV arthritis have high antibody titers to TM monomer and oligomers.

MAb CAE92A1. Antibodies were made by fusing X63.ag8.653 murine myeloma cells with spleen cells from BALB/c mice (14) immunized with purified CAEV-63 (7, 12). The CAEV-63 isolate was used in all experiments in this paper. Hybridoma supernatants were screened for reactivity to CAEV by solid-phase radioimmunoassay and to infected cells by immunofluorescence assay (28). One hybridoma-making antibody reacting with CAEV in both assays was cloned twice, and the immunoglobulin G2a monoclonal antibody (MAb) was designated CAE92A1. In immunoprecipitation reactions of ^{125}I-surface-labeled (22, 33) CAEV (data not shown) and [^{35}S]methionine metabolically labeled CAEV proteins (19, 22) with MAb CAE92A1, 38- and 90-kDa proteins were detected (Fig. 1, lane 5).

MAb CAE92A1 identifies TM. Absorptions were done to demonstrate that MAb CAE92A1 identified *env*-encoded TM and not SU. MAb CAE92A1 and isotype control MAb were reacted with [^{35}S]methionine-labeled CAEV proteins, and immune complexes were removed by adding protein G-coated Sepharose beads (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) and then by centrifugation. The proteins remaining in the supernatant were evaluated in immunoprecipitation reactions (20). MAb CAE92A1 absorption, but not isotype control MAb, removed the 38- and 90-kDa proteins recognized by serum antibodies from CAEV-infected goat 85G27 (6) (Fig. 1; compare lanes 2, 8, and 13) and from rWR-63-immunized goat 89G65 (25, 26) (Fig. 1; compare lanes 4, 9, and 14). However, antibodies from infected goat 85G27 recognized p28 and SU in MAb CAE92A1-absorbed antigen (Fig. 1, lane 8), and antibodies from rWR-63-immunized goat 89G65 recognized SU (Fig. 1, lane 9). Also, absorption of CAEV proteins with monospecific goat antiserum to SU (2) did not remove the 38- and 90-kDa proteins (data not shown). Since the 38- and 90-kDa proteins were antigenically distinct from SU and were recognized by antibodies to proteins encoded by the CAEV-63 *env* gene with a single open reading frame for SU and TM (25), it was concluded that they were TM proteins.

Oligomerization of TM. In addition to the 38- and 90-kDa proteins recognized by MAb CAE92A1 in immunoprecipitation reactions, the MAb bound 120-, 170-, and 210-kDa proteins in immunoblots (data not shown) of CAEV and CAEV antigens affinity purified (29) with MAb CAE92A1. Because the CAEV *env* TM region is too small to encode 90-kDa and larger proteins (25), oligomerization of TM was evaluated. Dissociating agents, including those dissociating human immunodeficiency virus (HIV) (35, 37) and simian immunodeficiency virus (37), TM oligomers did not dissociate the 90- and 120-kDa proteins recognized by MAb CAE92A1. However, these proteins were shown to be TM oligomers by dissociation as follows. Purified CAEV was incubated at 37°C for 15 min with 1% Nonidet P-40, 1% Triton X-100, and 2% β-mercaptoethanol; disrupted virus was mixed with 0.1 M glycine HCl (pH 2.8), containing 1 M NaCl and boiled for 5 min. Immunoblotting of treated virus

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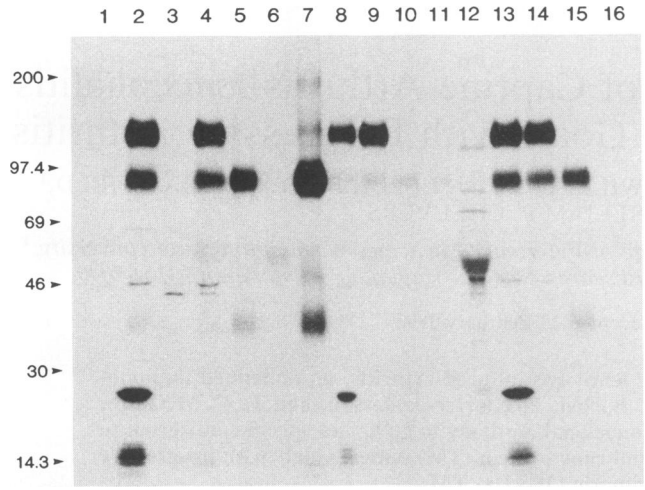

FIG. 1. Immunoprecipitation reactions of [^{35}S]methionine-labeled CAEV proteins absorbed with MAb CAE92A1. Proteins were unabsorbed (lanes 1 to 6), absorbed with MAb CAE92A1 (lanes 8 to 11), absorbed with isotype control MAb (lanes 13 to 16). Primary antibodies were sera from normal goat 85G05 (lane 1), from CAEV-infected goat 85G27 (lanes 2, 8, and 13), from rWR-SC11-immunized goat 89G62 (lane 3), and from rWR-63-immunized goat 89G65 (lanes 4, 9, and 14); MAb CAE92A1 (lanes 5, 10, and 15); and isotype control MAb (lanes 6, 11, and 16). Lane 7 contained CAEV proteins eluted from the MAb CAE92A1 absorption matrix; lane 12 had eluate from the isotype control MAb absorption matrix. Molecular masses (in kilodaltons) of ^{14}C-labeled protein standards appear on the left.

used previously described procedures (32), except that incubation of nitrocellulose with dilutions of serum from an infected goat or MAb was followed by the appropriate conjugate of horseradish peroxidase and anti-immunoglobulin antibodies (Kirkegaard & Perry, Gaithersburg, Md.). Immunoblots were developed with enhanced chemiluminescence reagents (4) and exposed to Hyperfilm-enhanced chemiluminescence autoradiography film (Amersham Corp., Arlington Heights, Ill.). Virus treatment caused the 90- and 120-kDa proteins recognized by MAb CAE92A1 and antibodies from an infected goat to decrease, while the 38-kDa monomer increased (Fig. 2). Also, MAb CAE92A1 reacted better with TM oligomers than with monomer (Fig. 2). Demonstration of CAEV TM oligomers is similar to that of HIV type 1 (HIV-1) TM (gp41), which forms oligomers of 120- and 160-kDa (35), and to those of HIV-2 and simian immunodeficiency virus SIVmac TM, which exist as homodimers (37).

CAEV TM lacks leucine zipper-like repeats. It is proposed that TM oligomerization in some retroviruses results from a coiled coil structure involving leucine zipper-like repeats (15). The CAEV TM sequence (25, 38) (in EMBL and GenBank data libraries, accession no. M60855) was examined for leucine zipper-like repeats of leucine or isoleucine residues separated by six amino acids (15). CAEV TM lacked these repeats and the sequence QNRRGLDLL that is conserved among some distantly related retroviruses (9) and used to align leucine zipper motifs (15). The cause of CAEV TM oligomerization is unknown; however, leucine zipper-like repeats are not required.

Glycosylation of TM. To verify that the 90-kDa TM protein identified by MAb CAE92A1 was glycosylated and that it was the same as gp90 (22), immunoprecipitation reactions

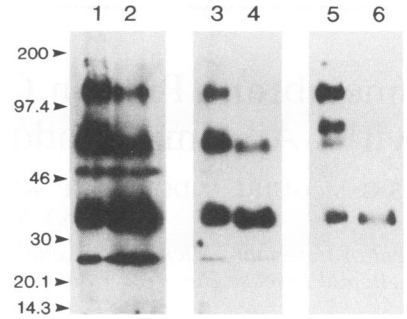

FIG. 2. Immunoblot of monomeric and oligomeric CAEV TM. Lanes 1, 3, and 5 contained untreated CAEV proteins, and lanes 2, 4, and 6 contained CAEV proteins treated to dissociate oligomers. Lanes 1 and 2 were reacted with a 1:100 dilution and lanes 3 and 4 were reacted with a 1:1,000 dilution of serum from an infected goat, and lanes 5 and 6 were reacted with MAb CAE92A1. Molecular masses (in kilodaltons) of stained standards (Rainbow standards; Amersham Corp.) appear on the left.

were done with [^{14}C]glucosamine-labeled CAEV proteins. Both MAb CAE92A1 (Fig. 3, lane 3) and antibodies from a CAEV-infected goat (Fig. 3, lane 1) identified gp90. The intensity of gp90 bands was low following a 4-month film exposure (Fig. 3), and a glycosylated 38-kDa protein was not detected. The relative intensities of SU and gp90 bands (Fig. 3, lane 1) are consistent with 21 SU and 4 TM predicted glycosylation sites (25, 38). In this report, the CAEV-63 SU was 115 kDa and the TM monomer was 38 kDa, predicting a glycosylated precursor of 153 kDa, consistent with the reported size of 150 kDa (8). A 45-kDa glycoprotein is predicted for CAEV TM (25, 38); however, the 38-kDa recognized by MAb CAE92A1 in other experiments suggests that not all of the possible sites are glycosylated.

TM immunodominant in goats with progressive arthritis. To verify that TM monomer and oligomers were immunodominant in goats with progressive arthritis, 10-fold dilutions of sera from four goats with progressive arthritis taken 5 years after CAEV-63 infection (6) were reacted in immunoblots with CAEV antigens. The immunoblot procedure was the same as that described in the preceding section on

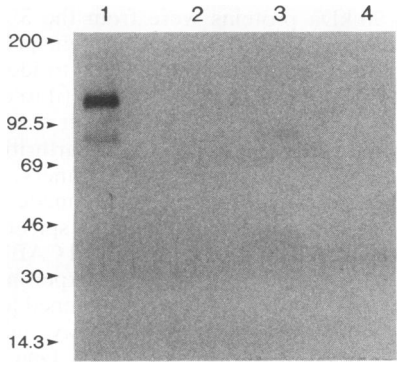

FIG. 3. Incorporation of [^{14}C]glucosamine into CAEV TM. Lanes 1 to 4 were glycosylated proteins bound in immunoprecipitation reactions by primary antibodies including sera from infected goat 81G18 (lane 1) and from control goat 81G13 (lane 2), MAb CAE92A1 (lane 3), and isotype control MAb (lane 4). Molecular masses (in kilodaltons) of ^{14}C-labeled protein standards appear on the left.

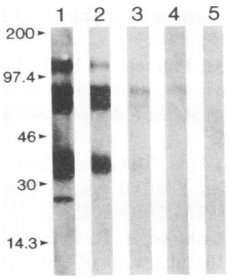

FIG. 4. Immunoblot serum antibody titers to CAEV TM from a goat with progressive CAEV arthritis. The antigens in all lanes were CAEV proteins. Primary antibodies in lanes 1 to 4 were 10-fold dilutions (starting at 1:100) of serum from infected goat 85G28; for lane 5, a 1:100 dilution of serum from control goat 85G06 was used. Molecular masses (in kilodaltons) of stained standards (Rainbow standards; Amersham Corp.) appear on the left.

oligomerization of TM. Antibody titers to monomeric (38-kDa) and oligomeric (90- and 120-kDa) TM proteins were very high in each goat serum, ranging from 1:10,000 to 1:100,000 (example in Fig. 4). However, antibody titers to p28, the major core protein, were at least 10-fold lower. Sera from two age-matched control goats did not react with CAEV proteins in immunoblots (example in Fig. 4). Two of four goats with progressive arthritis had higher antibody titers to oligomeric than to monomeric TM, and MAb CAE92A1 also preferentially reacted with oligomeric TM. These results are similar to the observation that some human MAbs to HIV-1 TM preferentially recognize oligomeric over monomeric TM (35).

The CAEV TM form predominating in virus or infected cells in arthritic joints (23) is unknown; however, any TM could contribute to inflammation following reaction with antibody and other immune system components. Identification of immunodominant CAEV proteins as SU and TM is similar to observations for other lentiviruses (3, 18, 34) and allows identification of immunodominant epitopes. Antigenic variants of CAEV isolated from cells (17, 31) and cell-free synovial fluid (6, 30) from arthritic goats result from changes of neutralization-sensitive epitopes (30), some of which are on SU (26). However, CAEV antigenic variants have considerable sequence homology (25, 36, 38) and likely share several SU and TM epitopes. If immune responses to immunodominant SU and TM epitopes shared among antigenic variants (defined by antibody neutralization in vitro) are important determinants of arthritis, specific modulation of these immune responses may prevent arthritis.

We thank Kathleen Reeve and Linda Norton for technical assistance.

This work was supported by grants from the National Institutes of Health (NIAMSD AR27680 and NCI 50141) and the Medical and Veterinary Biotechnology Center, Washington Technology Centers.

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