JOURNAL OF VIROLOGY, May 1990, p. 2396–2398
0022-538X/90/052396-03$02.00/0

Vol. 64, No. 5

Severity of Arthritis Is Predicted by Antibody Response to gp135 in Chronic Infection with Caprine Arthritis-Encephalitis Virus

DONALD KNOWLES, JR.,[1]* WILLIAM CHEEVERS,[2] TRAVIS McGUIRE,[2] THERESA STEM,[2] AND JOHN GORHAM[1]

Animal Disease Research Unit, U.S. Department of Agriculture–Agricultural Research Service, Pullman, Washington 99164-7030,[1] and Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040[2]

Received 19 June 1989/Accepted 8 January 1990

Antibody titers to caprine arthritis-encephalitis virus surface glycoprotein gp135 and core protein p28 in synovial fluid and serum from 35 goats infected for 3 years were compared with the histologic severity of arthritis in these animals. Anti-gp135 antibody titers in synovial fluid and serum directly reflect the severity of carpal arthritis in chronically infected goats.

Caprine arthritis-encephalitis virus (CAEV), a lentivirus, causes progressive arthritis in goats (1, 6, 7). The role of CAEV in the pathogenesis of arthritis was previously investigated in a study conducted for 18 months following intra-articular and intravenous inoculation. That study demonstrated a positive association between the isolation of CAEV from the synovial cavity and clinical arthritis measured by carpal swelling (12). Two previous studies have implicated CAEV-specific immune responses in the pathogenesis of carpal arthritis. The first study evaluated synovial fluid 6 months after intra-articular and intravenous CAEV inoculation and revealed a marked immunoglobulin G1 response in synovial fluid concurrent with preferential antibody reactivity to CAEV gp135 (10, 11). In the second study, goats challenged with CAEV during persistent CAEV infection or after vaccination with inactivated virus developed more severe arthritis than uninfected or nonvaccinated goats did (13). In this report, quantitation of the antibody response to gp135 and p28 demonstrates that the severity of chronic arthritis induced by oral infection with either of two biologically cloned isolates of CAEV is directly related to antibody titers to the surface glycoprotein gp135.

To investigate the relationship between CAEV-specific antibody responses and arthritis, 35 goat kids from a CAEV-free Saanen breeding herd were orally infected as previously described (3) with either CAEV(CO) (5, 15) (18 kids) or CAEV(63) (6) (17 kids). Serum and carpal joint synovial fluid were collected prior to necropsy at 3 years postinfection. Radiocarpal joint synovial membrane was fixed in 10% buffered Formalin, paraffin embedded, sectioned at 4 to 6 μm, and stained with hematoxylin and eosin for histologic examination. Radiocarpal joint lesion grades were based on the distribution of inflammatory cells in the synovial membrane and subsynovial stroma (3). Grade 0 was a normal synovial membrane. Grade 1 lesions contained scattered accumulations of lymphocytes, plasma cells, and macrophages within areas of synovial membrane hyperplasia. Grade 2 lesions were similar to grade 1 lesions, except that multifocal accumulations of inflammatory cells extended into the subsynovial stroma and there were occasional areas of synovial cell necrosis. Grade 3 lesions contained coalescing synovial and subsynovial accumulations of inflammatory cells, with a tendency toward lymphoid-nodule formation and multifocal areas of necrosis. The 70 carpal synovial membranes from the 35 goats had the following lesion grades: 9 grade 0, 24 grade 1, 16 grade 2, and 21 grade 3. Antibody titers to CAEV gp135 and p28 were determined by immunoprecipitation with serum or carpal synovial fluid as the source of antibody and radiolabeled infected or uninfected caprine synovial membrane cells as the source of antigen. Immune complexes were precipitated with Formalin-treated staphylococci bearing protein A (Pansorbin; Calbiochem-Behring) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (4, 9). Antibody titers were determined visually from autoradiographs after 14 days of exposure and were expressed as the highest dilution of serum or synovial fluid retaining a positive signal corresponding to p28 and gp135.

Figures 1 and 2 show representative sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels of immunoprecipitates formed between serum and synovial fluids of goats and CAEV antigen. Since undiluted serum and synovial fluid were not tested, an endpoint of 0 represents an antibody titer of less than 10^{-1}. Controls included serum and synovial fluid from CAEV-negative goats (G11, G5), serum from a goat hyperimmunized with CAEV (G19), and antigen prepared from uninfected caprine synovial membrane cells. As expected, G5 synovial fluid and G11 serum did not react with labeled antigens (Fig. 1 and 2), and G19 serum precipitated labeled proteins from the viral antigen preparation but not the control antigen preparation (Fig. 2). Serum and synovial fluids from CAEV-infected goats did not react with labeled proteins from the control antigen preparation (Fig. 2) and staphylococci bearing protein A did not precipitate labeled control antigen or labeled CAEV antigen (not shown). Right carpal synovial-fluid dilutions from five goats are represented in Fig. 1. The anti-gp135 antibody endpoints are 10^{-1}, 10^{-2}, 10^{-1}, 10^{-3}, and 10^{-2}, and the anti-p28 antibody endpoints are 0, 10^{-2}, 0, 10^{-2}, and 10^{-2}. Dilutions of serum and contralateral synovial fluids from the same goat are shown in Fig. 2. The serum and left and right synovial-fluid anti-gp135 antibody endpoints are 10^{-2}, 10^{-1}, and 10^{-2}, and the anti-p28 antibody endpoints are 10^{-2}, 0, and 10^{-2}.

Tables 1 and 2 show the distribution of carpal synovial lesions and anti-gp135 and anti-p28 antibody titers in synovial fluid and serum for all goats. Anti-gp135 antibody titers in synovial fluid more accurately predict the presence and severity of carpal synovial lesions than do anti-gp135 titers in

* Corresponding author.

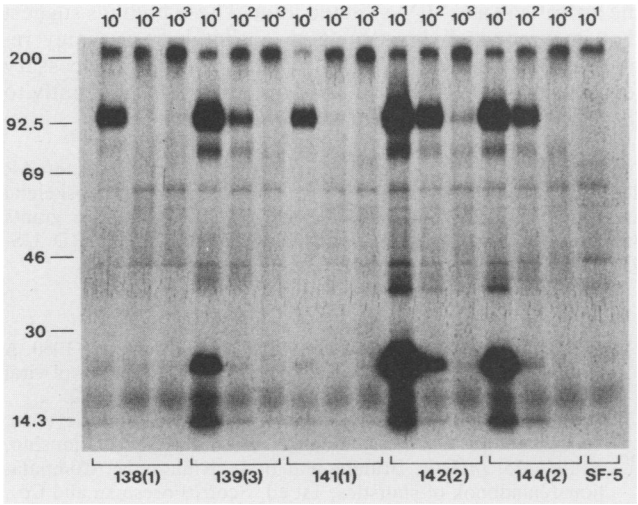

FIG. 1. Immunoprecipitations of [^{35}S]methionine- and [^{3}H]glucosamine-labeled CAEV proteins. Right synovial fluids of five CAEV-infected goats and synovial fluid from CAEV-negative goat 5 were used. The synovial fluid dilution is indicated at the top of each lane. The goat number and lesion grade (in parentheses) are presented below the appropriate lanes. The electrophoretic mobility of marker proteins is indicated in kilodaltons.

serum (Table 1). An anti-gp135 titer in synovial fluid of 10^3 (22 joints) was always associated with carpal arthritis. However, whereas relatively high titers of anti-gp135 antibody in synovial fluid (10^2 to 10^3) were associated with mild lesion grades (0 or 1) in 12 joints, anti-gp135 antibody levels of 10^2 to 10^3 in serum were associated with mild lesion grades (0 or 1) in 28 joints (Table 1). Therefore, anti-gp135 antibody titers in synovial fluid more accurately predict mild carpal lesions (0 or 1) than do anti-gp135 antibody titers in serum. The most

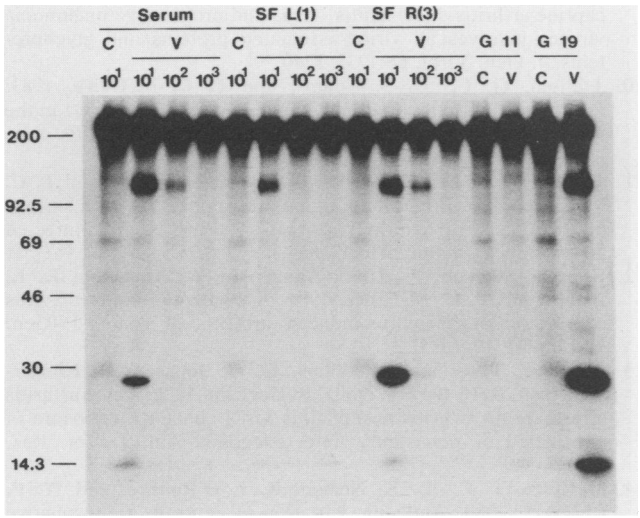

FIG. 2. Immunoprecipitations of [^{35}S]methionine- and [^{3}H]glucosamine-labeled CAEV proteins by serum and contralateral synovial fluids of goat 139. The serum or synovial fluid (SF) dilution is presented at the top of each lane. The lesion grades for the left and right radiocarpal joints are in parentheses. Abbreviations: C, control antigen; V, CAEV antigen. The electrophoretic mobility of marker proteins is indicted in kilodaltons.

TABLE 1. Anti-gp135 antibody titers in synovial fluid and serum of goats with chronic CAEV arthritis

Lesion grade	No. of joints	Distribution of lesions among anti-gp135 antibody titers in:							
		Synovial fluid[a]				Serum[b]			
		10^3	10^2	10^1	0	10^3	10^2	10^1	0
3	21	14	7	0	0	17	4	0	0
2	16	5	10	1	0	14	1	1	0
1	24	3	7	14	0	7	15	2	0
0	9	0	2	3	4	4	2	3	0

[a] Number of synovial fluid samples with the indicated anti-gp135 antibody titer and the corresponding lesion grade.
[b] Number of carpal joints with the indicated lesion grade from goats with the corresponding serum anti-gp135 antibody titer.

likely factor contributing to this finding is the distribution of CAEV among several organ systems (3). The expression of CAEV in lymph nodes, mammary gland, and other synovial spaces would contribute to anti-gp135 antibody titers in serum. The resulting antibody would reflect contributions from multiple sites of CAEV expression, although it may not reflect mild lesions in a synovial space (Table 1).

The data in Table 2 indicate that anti-p28 antibody titers in synovial fluid predict the presence or severity of carpal synovial lesions less accurately than do anti-gp135 antibody titers in synovial fluid or serum. An anti-p28 antibody titer in synovial fluid of 10^2 accurately predicted the presence of carpal arthritis. However, anti-p28 antibody titers in synovial fluid of 0 or 10^1 were not accurate indicators of mild lesions or the absence of lesions, since these titers were associated with lesion grade 2 or 3 in 25 joints (Table 2). Anti-p28 antibody titers in serum poorly predicted the presence or severity of carpal arthritis (Table 2). As was the case for anti-gp135 antibody in serum, anti-p28 antibody in serum was inaccurate in predicting mild lesion grades. An anti-p28 titer in serum of 10^2 was associated with mild lesions (grade 0 or 1) in 19 joints. Additionally, anti-p28 antibody titers in serum of 0 or 10^1 were associated with lesion grades 2 or 3 in 14 joints. This is in marked contrast to anti-gp135 antibody titers in synovial fluid or serum of 0 or 10^1, which were associated with a joint lesion grade of 2 or 3 in only one joint (Table 1). The decreased relationship between anti-p28 antibody titers and lesion severity compared with anti-gp135 antibody titers and lesion severity is most probably the result of the preferential antibody response to CAEV gp135 compared with p28 (11). This response establishes a greater concentration of anti-gp135 antibody than anti-p28 antibody

TABLE 2. Anti-p28 antibody titers in synovial fluid and serum of goats with chronic CAEV arthritis

Lesion grade	No. of joints	Distribution of lesions among anti-p28 antibody titers in:					
		Synovial fluid[a]			Serum[b]		
		10^2	10^1	0	10^2	10^1	0
3	21	8	9	4	11	9	1
2	16	4	9	3	12	2	2
1	24	1	10	13	15	8	1
0	9	0	2	7	4	5	0

[a] Number of synovial fluid samples with the indicated anti-p28 antibody titer and the corresponding lesion grade.
[b] Number of carpal joints with the indicated lesion grade from goats with the corresponding serum anti-p28 antibody titer.

TABLE 3. Summary of chi-square statistics and contingency coefficients[a] for the dependence of arthritis severity on anti-gp135 and anti-p28 antibody titers

Antibody	Sample	χ^2	Contingency coefficient	Significance	Alpha
gp135	Synovial fluid	62.7 (9 df)	0.69	0.999	0.001
gp135	Serum	27.9 (6 df)	0.53	0.999	0.001
p28	Synovial fluid	19.4 (6 df)	0.47	0.995	0.005
p28	Serum	6.99 (6 df)	0.30	0.50–0.75	0.25–0.50

[a] Contingency coefficient (CC) is calculated from the χ^2 values and the total number of observations: $CC = [\chi^2/(\chi^2 + 70)]^{1/2}$.

(11). Assuming that the decay of antibodies to gp135 and p28 is similar, anti-gp135 antibody may remain detectable during periods of viral inactivity, while anti-p28 antibody decays below detectable levels.

A summary of the statistical analysis of the data in Tables 1 and 2 is shown in Table 3. The null hypothesis that carpal lesion grades and anti-CAEV antibody levels in synovial fluid or serum were independent variables was tested by chi-square statistics. Chi-square values of 62.7 and 27.9 were obtained for anti-gp135 antibody levels in synovial fluid and serum, respectively, indicating that lesion grades and anti-gp135 antibody titers were dependent variables at a significance of 0.999. Chi-square values of 19.4 and 6.99 were obtained for comparisons of lesion grades and anti-p28 antibody titers in synovial fluid and serum, respectively. These values were significant at 0.995 and 0.500. Thus, anti-p28 antibody titers in synovial fluid and carpal lesion severity were dependent variables, although at a lesser significance than anti-gp135 antibody and lesion severity. Anti-p28 antibody levels in serum and lesion severity are poorly related. The contingency coefficient (2), which indicates the extent of the relationship between lesion severity and anti-CAEV antibody levels, demonstrated that anti-gp135 antibody levels in synovial fluid and lesion severity were most closely related, whereas anti-p28 antibody levels in serum and lesion severity were the least related.

Recent work with the same goats described in the present study demonstrated that the presence and severity of carpal arthritis is dependent in part on the initial infecting CAEV isolate (3). Results from the present study demonstrate that regardless of the infecting isolate, carpal arthritis is related to titers of anti-gp135 antibody. In addition to the potential contributions of virus-specific antibody in inducing arthritis, virus-specific neutralizing antibodies may play a role in the recurrence of arthritis (8, 14). Neutralizing antibody occurs at low levels and is slow to develop (14). However, virus isolates expressing antigenically variable neutralization-sensitive epitopes arise in joint fluid during chronic CAEV arthritis (8, 14) and may contribute to periods of inflammation and recurrent joint swelling (3, 14).

In summary, collective data indicate that CAEV arthritis is an immunopathologic process in which specific antibody and sensitized lymphocytes react with CAEV antigen in the synovial cavity. The expression of virus or viral genes is accompanied by a vigorous immune response, which includes a slowly developing neutralizing-antibody component. The failure of this immune response to eliminate all latently infected cells and the formation of antigenic variants which escape neutralizing antibody leads to recurrent expression of CAEV. The interaction of expressed virus with the immune response is wholly or partly responsible for the arthritis in a CAEV-infected joint. These findings suggest that immunoprophylaxis for this lentiviral disease may require vaccination with CAEV epitopes which are necessary for virus neutralization and do not contribute significantly to the immunopathogenesis of inflammation.

This work was supported by Public Health Service grant AR 27680 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and U.S. Department of Agriculture grants 85-CRSR-1-1731, 88-34116-3735, 58-AHZ-2-679, and BARD US-937-85.

LITERATURE CITED

1. **Adams, D. S., T. B. Crawford, and P. Klevjer-Anderson.** 1980. A pathogenetic study of the early connective tissue lesions of viral caprine arthritis-encephalitis. Am. J. Pathol. **99**:257–272.
2. **Bruning, J. L., and B. L. Kintz.** 1968. Nonparametric tests, miscellaneous tests of significance, and indices of relationship, p. 195–215. *In* J. L. Bruning and B. L. Kintz (ed.), Computational handbook of statistics, 1st ed. Scott, Foresman and Co., Atlanta.
3. **Cheevers, W. P., D. P. Knowles, T. C. McGuire, D. R. Cunningham, D. S. Adams, and J. R. Gorham.** 1988. Chronic disease in goats orally infected with two isolates of the caprine arthritis-encephalitis lentivirus. Lab. Invest. **58**:510–517.
4. **Cheevers, W. P., T. A. Stem, D. P. Knowles, and T. C. McGuire.** 1988. Precursor polypeptides of caprine arthritis-encephalitis lentivirus structural proteins. J. Gen. Virol. **69**:675–681.
5. **Clements, J. E., O. Narayan, and L. C. Cork.** 1980. Biochemical characterization of the virus causing leukoencephalitis and arthritis in goats. J. Gen. Virol. **50**:423–427.
6. **Crawford, T. B., D. S. Adams, W. P. Cheevers, and L. C. Cork.** 1980. Chronic arthritis in goats caused by a retrovirus. Science **207**:997–999.
7. **Crawford, T. B., D. S. Adams, R. D. Sande, J. R. Gorham, and J. B. Henson.** 1980. The connective tissue component of the caprine arthritis-encephalitis syndrome. Am. J. Pathol. **100**: 443–450.
8. **Ellis, T. M., G. E. Wilcox, and W. F. Robinson.** 1987. Antigenic variation of caprine arthritis-encephalitis virus during persistent infection of goats. J. Gen. Virol. **68**:3145–3152.
9. **Gogolewski, R. P., D. S. Adams, T. C. McGuire, K. L. Banks, and W. P. Cheevers.** 1985. Antigenic cross-reactivity between caprine arthritis-encephalitis, visna and progressive pneumonia viruses involves all virion-associated proteins and glycoproteins. J. Gen. Virol. **66**:1233–1240.
10. **Johnson, G. C., D. S. Adams, and T. C. McGuire.** 1983. Pronounced production of polyclonal immunoglobulin G1 in the synovial fluid of goats with caprine arthritis-encephalitis virus infection. Infect. Immun. **41**:805–815.
11. **Johnson, G. C., A. F. Barbet, P. Klevjer-Anderson, and T. C. McGuire.** 1983. Preferential immune response to virion surface glycoproteins by caprine arthritis-encephalitis virus-infected goats. Infect. Immun. **41**:657–665.
12. **Klevjer-Anderson, P., D. S. Adams, L. W. Anderson, K. L. Banks, and T. C. McGuire.** 1984. A sequential study of virus expression in retrovirus-induced arthritis of goats. J. Gen. Virol. **65**:1519–1525.
13. **McGuire, T. C., D. S. Adams, G. C. Johnson, P. Klevjer-Anderson, D. D. Barbee, and J. R. Gorham.** 1986. Acute arthritis after caprine arthritis-encephalitis virus challenge exposure of vaccinated or persistently infected goats. Am. J. Vet. Res. **47**:537–540.
14. **McGuire, T. C., L. K. Norton, K. I. O'Rourke, and W. P. Cheevers.** 1988. Antigenic variation of neutralization-sensitive epitopes of caprine arthritis-encephalitis lentivirus during persistent arthritis. J. Virol. **62**:3488–3492.
15. **Narayan, O., J. E. Clements, J. D. Strandberg, L. C. Cork, and D. E. Griffin.** 1980. Biological characterization of the virus causing leukoencephalitis and arthritis in goats. J. Gen. Virol. **50**:69–79.