77

BARD

FINAL REPORT

PROJECT NO. US'937-85

Subunit Vaccine for the Retrovirus of Caprine Arthritis-Encephalitis and Studies on its Pathogenesis and Epidemiology

W.P. Cccheevers, K. Perk, T.C. McGuire, N. Safran,
A. Shimshony, D.S. Adams, I. Hod

1990

Standard BARD Cover Page for Scientific Reports

Date: July 3, 1990

12 -07- 1990

BARD
P.O. BOX 6
Bet Dagan, ISRAEL

BARD project No. US-937-85R

Title

Subunit vaccine for the retrovirus of caprine arthritis-
encephalitis and studies of its pathogenesis and epidemiology

Investigators' Names (Principal listed first)

William P. Cheevers

Kalman Perk

Investigators' Institutions

Washington State University

Pullman, Washington U.S.A.

Koret School of Veterinary

Medicine, Rehovot, Israel

Project's starting date: September 17, 1986

Type of Report: 1st Annual 2nd Annual Final X

Signature
Principal Investigator

William P. Cheevers

Signature
Institution's Authorizing Official

Robert V. Smith

Abstract

The env gene encoding the envelope glycoprotein gp135 of two antigenic variants of CAEV has been cloned, sequenced and expressed in vaccinia virus. Recombinant vaccinia express a gene product that (1) comigrates in polyacrylamide gel electrophoresis with native gp135, (2) reacts in immunoprecipitation and Western blot assays with sera from goats chronically infected with CAEV, (3) inhibits the ability of immune goat serum to neutralize virus, and (4) induces neutralizing antibody in vaccinated goats. An indirect ELISA using biotin-avidin amplification was developed to detect antibodies to CAEV in goat sera. Seroepidemiological surveys using this test with RIA and AGID established that the prevalence of CAEV infection among imported Saanen herds in Israel is similar to that in the U.S.A. (80-85%), whereas local Beduin herds not in contact with European breeds are negative. Herd management experiments indicate that the prevalence of CAEV in Israel as well as in the U.S.A. can be significantly reduced by pasteurization and segregation practices. In a pathogenesis study, newborn Saanen goats were inoculated orally with biologically cloned CAEV, and the development of immune responses and disease was followed for three years. Predominant lesions were chronic arthritis and mastitis. Arthritis developed with a progressive course which may be promoted by recrudescent inflammation caused by periodic emergence of antigenic variants of CAEV. Acute inflammation of synovial membrane is accompanied by clinical enlargement of joints, increased volume and cellularity of synovial fluid, and increased titers of anti-gp135 antibody in serum and synovial fluid. An electron microscopic study also revealed the presence of CAEV in brain sections of diseased goats. Nucleotide sequence data reveal that the genetic organization of CAEV is similar to ovine, feline, bovine and human lentiviruses, with significant sequence homology being confined mainly to gag and pol genes.

Objectives of the Original Research Proposal

Objectives were as follows:

1. Production of a subunit vaccine from protein(s) containing neutralizing determinants of CAEV
2. Perform a seroepidemiologic study to define CAEV infection in Israel
3. Define epidemiological relationships that may modify expression and/or aid in control of the spread of disease
4. Further study of the pathogenesis of CAEV, its host range, the genomic relatedness of CAEV to other isolates and other relevant retroviruses, including HIV

Final Report

Objective 1: CAEV subunit vaccine based on neutralizing determinants

At the time of submission of the original proposal, our data on CAEV neutralization indicated that sera of chronically infected goats failed to neutralize CAEV even though antibody bound to the virion surface. A rabbit antiserum was prepared that neutralized CAEV in a complement-dependent reaction in vitro, and successive virus isolates from infected goats did not exhibit antigenic variation of epitopes sensitive to this antiserum. Subsequent re-evaluation of this question using a virus titer reduction assay revealed the presence of complement-independent neutralizing antibody in the sera of some persistently-infected goats and demonstrated antigenic variation of virion-associated, neutralization-sensitive epitopes during chronic CAEV infection (McGuire et al., 1988; enclosed).

To evaluate neutralization epitopes for a potential vaccine, we have cloned and sequenced the env gene encoding the virion gp135 surface glycoprotein of CAEV(Co) and CAEV(63), two antigenic variants of CAEV defined by serum neutralization (McGuire et al., 1988). The CAEV(Co) sequence was derived from three subclones of the CAEV(Co) provirus, designated pCo-1.9, pCo-2.4 and pCo-2. The CAEV(63) sequence was derived from three cDNA clones constructed from poly A⁺ RNA extracted from cells infected with CAEV(63). Figure 1 shows these clones in relation to the CAEV env open reading frame. Sequences were analyzed by computer to evaluate structure, genetic variation and distribution of variable neutralization epitopes. Analysis of the deduced amino acid sequence of the CAEV primary env gene product identifies a glycoprotein precursor with three major hydrophobic domains, corresponding to an unusually large 9.4 kilodalton amino-terminal signal peptide, an Arg-Lys-Lys-Arg outer membrane-transmembrane processing site and a membrane spanning anchor domain within the transmembrane region. This general structure is similar to other lentivirus envelope glycoprotein precursors. The env genes of these CAEV isolates exhibit 89% nucleotide sequence homology. Genetic variation includes relatively short regions of sequence addition, deletion and substitution and numerous single base substitutions encoding nonconservative amino acid changes mainly in the outer membrane region of the gp135.

Recombinant vaccinia viruses were derived between WR vaccinia and pSC11 vaccinia shuttle plasmids containing the CAEV(Co) and CAEV(63) env genes (Figs. 2 and 3). The recombinant proteins expressed by vaccinia comigrate in polyacrylamide gel electrophoresis with native CAEV gp135 and react in immunoprecipitation reactions with sera of goats chronically infected with CAEV. Native CAEV gp135 is monospecifically immunoprecipitated by serum of rabbits and goats immunized with recombinant vaccinia. Expression of

variable neutralization eptopes on recombinant gp135 was demonstrated by two methods: (1) Recombinant gp135 inhibits neutralization of CAEV by serum of infected goats (Table 1). (2) Serum of goats vaccinated with recombinant vaccinia neutralizes CAEV (Tables 2 and 3). Data based on heterologous blocking experiments (Table 1) and neutralization assays (Table 3) indicate that neutralization epitopes exhibit both isolate-specific and broader reactivities.

Objective 2: Diagnostic test evaluation and seroepidemiology of CAEV

An ELISA test for detection of CAEV antibodies in goat serum using sucrose gradient purified CAEV antigen and detection by biotin-avidin amplification was developed. This assay was tested using 520 serum samples, mainly from California herds, and compared to agar gel immunodiffusion (AGID) and radioimmunoassay (RIA) with CAEV p28 antigen. These results indicated that the ELISA test with CAEV antigen is comparable to RIA and more sensitive than the commonly used AGID test (Archambault et al., 1988; enclosed).

Monoclonal antibodies recognizing various CAEV structural antigens were developed for use in an assay to detect CAEV in the milk of infected goats. Detection of bound antigen utilized monoclonal antibody conjugated to horseradish peroxidase, followed by three washes and a subsequent incubation with substrate. Table 4 shows a sample of results obtained with cow milk and milk of infected and uninfected goats. Note that the signals using infected goat milk are higher than the signal obtained using cow or uninfected goat milk.

Although the first Israeli CAEV isolation was made in 1981, the prevalence and incidence of CAEV infection and disease in Israel were unknown prior to initiation of this project. For many generations goats have played an

important role in Israel's agriculture. The diversity of goat farming in Israel is widespread and ranges from primitive management to intensive farming for milk and cheese, the latter being a new and expanding industry in Israel. This seroepidemiological study examined 1426 serum samples obtained from selected goat herds with intensive farming (high milk production in moshavim and kibbutzim) and from Beduin and local Arab village herds. A pilot study was conducted using small random groups of goats obtained from 17 herds representing two breeds and management practices: Beduin and local Arab breed from four different villages and 13 herds of imported Saanens from different moshavim with intensive milk production. Serum samples were tested by ELISA, RIA and AGID. Results in Table 5 show a good correlation between ELISA AND RIA. AGID was much less sensitive, and only sera with high titers were positive using this test. Note that the local breed was negative in all cases where these goats were isolated, without contact with imported European breeds. In contrast, the Saanen herds showed an average of 85% seroconversion for CAEV antibody. Clinical arthritis and other manifestations of CAE disease were present on these farms, and at the time of screening, none used pasteurized colostrum, and kids were kept with their dams. Similar results were obtained with sera from many other Israeli herds (Table 6). Results not presented show that seroconversion increased after cross breeding of the local goats with Saanens.

Objective 3: Epidemiological relationships and disease control

In this project we studied the relationship between farm management procedures and CAEV infectivity in Israel. In one experiment 70 newborn kids were divided into three groups. Twenty kids in the first group were fed bovine colostrum and milk; thirty kids in the second group were fed

pasteurized goat colostrum; twenty kids in the third group were fed unpasteurized goat colostrum and milk. Table 7 shows the serology results obtained for these three groups at one year of age. Another study extended this experiment by testing the effect of pasteurization of goat colostrum and milk on five different farms in different areas of the country. The serological results obtained from 322 one year old does randomly selected from the five farms are shown in Table 8.

The serological data from these two experiments indicate the importance of pasteurization or feeding kids with cow milk. However, the importance of separation between the infected and seronegative animals is indicated by the following observations. Due to difficulties in management, goats receiving pasteurized and unpasteurized milk were placed together in one experiment. The incidence of CAEV seroconversion increased in this group from 9% to 54% within one year.

Our conclusions from experiments in objectives 2 and 3 are as follows: The serologic prevalence of CAEV in Israeli goat dairies of the Saanen breed is on the order of 80%, comparable to that in industrialized Western dairies, whereas infection is essentially absent in local Arab breeds. The goat dairies that reared kids on pasteurized milk had a lower seroprevalence than those that did not. A relationship between age and seroconversion was noted, indicating that continued horizontal contact transmission may be important to these dairies and limiting the effect of a pasteurization rearing program on the control of CAEV infection. For example, when kids at birth were fed cow colostrum and isolated until 12 months of age and then placed in contact with the herd, a large number became infected. In contrast, CAEV-negative kids fed cow colostrum and reared in strict isolation and separated from the rest of the herd by fencing show a very low incidence of CAEV infection.

Seroconversion data were supplemented by virus isolation studies. The first Israeli isolate was obtained in 1981 from an adult goat showing clinical signs and pathologic changes typical of advanced, progressive CAE disease. CAEV has been subsequently isolated from the joints and milk of several diseased goats from different herds and also from moribund kids. During the last two years, CAEV isolations have been made from peripheral blood. Twenty-nine isolations from dams and two from bucks have been obtained by cocultivation of centrifuged peripheral blood cells from CAE diseased or ELISA-positive goats with normal goat monocytes. It is noteworthy that no correlation was detected between positive virus isolations and level of serum antibodies or stage of disease. No isolations were made from seronegative goats.

Objective 4: CAEV pathogenesis, host range, and relatedness to other
lentiviruses

Pathogenesis

Newborn Saanen goats were orally infected with CAEV(63) or CAEV(Co), two distinct isolates of CAEV distinguishable by anti-p28 monoclonal antibodies and by isolate-specific neutralization epitopes. Development of antibodies to CAEV and lesions were followed in these goats for three years. The results have been published (Cheevers et al., 1988; enclosed). Briefly, all goats seroconverted for antibodies to CAEV, and there were no serologic reversions. Histologic lesions at three years postinfection consisted of chronic inflammation of variable incidence and severity in joints and mammary gland. Clinical arthritis correlated with severe inflammation of radiocarpal synovium. Statistical evaluations of clinical and histologic parameters indicated that chronic joint disease was significantly more frequent and more

severe in the group of goats infected with CAEV(63) than in the group infected with CAEV(Co). Analysis of the temporal development of arthritis during the first three years of infection indicated that the extent of eventual chronic disease in individual joints was apparent relatively early after infection, that joints with severe chronic lesions were clinically enlarged at irregular intervals, and that the pattern of recurrent arthritis was unique for each joint. Thus, chronic arthritis develops with a progressive course which may be promoted by recrudescent inflammation. Infectious virus was recovered three years after infection from selected tissues of 12 of 17

CAEV(63)-infected goats and 11 of 18 CAEV(Co)-infected goats. Virus recovery was independent of the virus isolate used for initial inoculation and did not correlate with the severity of lesions.

In an extension of this study (Knowles et al., 1990; enclosed), antibody titers to CAEV gp135 envelope glycoprotein and p28 core protein in synovial fluid and serum were compared with histologic severity of joint inflammation in these animals. Anti-gp135 antibody titers directly reflected the severity of carpal arthritis, regardless of the initial CAEV isolate used for initial inoculation.

In another study conducted in Israel, several brain and ganglionic tissue samples from six adult goats one to three years old that had developed CAE were randomly selected from two heavily afflicted herds and examined by electron microscopy. CAE was diagnosed at postmortem examination and confirmed by histopathology and serology. All showed marked proliferative synovitis with cartilage erosions and chronic interstitial pneumonia and were positive for CAEV antibodies by AGID and ELISA. Tissue samples from six representative healthy controls were also examined. Tissue sections were fixed in 3% glutaraldehyde in Millonig's buffer and post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer. After dehydration in ethanol and

propylene oxide, tissues were embedded in epon-araldite. Thin sections were stained in uranyl acetate and lead citrate. Typical intracytoplasmic type A retrovirus particles were seen in neural tissue of three diseased goats (Figs. 4-6). The electron micrographs in Figs. 4 and 5 illustrate selected samples of thin sections of a cluster of A particles spinal ganglia of the dorsal root. Particles observed in other ganglia are shown in Fig. 6. The intracytoplasmic A particles consisted of two electron dense shells of which the inner shell is more dense. Such particles, which may be precursors of the electron dense core of type B and D retrovirus particles, were also seen in lentivirus-infected cell cultures and in the tumor cells of spontaneous sheep pulmonary adenomatosis. In addition to A particles, there were a number of aberrant multilaminated particles. Such aberrant structures are also typical of maedi/visna and CAEV infected cells in culture. No particles were seen in control samples. It is noteworthy that budding particles, which are present in cell cultures productively infected with lentiviruses, were not seen in any of the goat tissue samples.

Host Range

The host range of CAEV was found not to be limited to domestic goats. CAEV antibodies were demonstrated in the sera of 5 of 18 gazelles, 2 of 5 ibex, 3 of 7 goat-ibex crosses, and 4 of 26 deer. Laboratory rodents, including nude mice inoculated with CAEV, did not develop CAEV antibodies.

Genetic relatedness

Restriction endonuclease maps and cross hybridization studies of the genome of several independent CAEV isolates as well as comparisons with maedi/visna isolates have now been completed. Although considerable restriction site heterogeneity has been noted between independent isolates, all CAEV isolates examined to date exhibit extensive nucleotide sequence homology by hybridization as well as substantial homology in the gag-pol

region with maedi/visna. These hybridization results have been confirmed by sequencing of at least two CAEV isolates. In addition, computer analysis of sequence has indicated significant pol gene homology among all lentiviruses examined, including HIV.

Description of Cooperation

Collaboration in this project between U.S. and Israeli investigators centered mainly around development of the ELISA assay for serodiagnosis of CAEV. The Israeli investigator visited Washington State University for collaborative development of CAEV antigen purification and ELISA plate binding assays, and worked in Israeli with reagents jointly developed in the U.S.

Evaluation of research achievements

Significant progress was made on all objectives. Development of a specific, sensitive and rapid ELISA test for CAEV antibodies in goat sera enabled investigators to evaluate the prevalence of CAEV in Israeli. This knowledge make it possible to institute meaningful management practices to control this disease with the attendant benefits of increased milk and cheese production, longevity and reproductive performance in Israeli dairy herds. Expression of recombinant CAEV antigen will make it possible to develop an ELISA test based on synthetic peptide antigens that avoids the expensive necessity for producing large amounts of viral antigen. Construction of recombinant vaccinia expressing the CAEV gp135 envelope glycoprotein and the ability of this construct to elicit neutralizing antibody are the first necessary steps to determine if neutralizing antibody is important in the development of a CAEV vaccine.

Manuscripts

1. McGuire, T.C., Norton, L.K., O'Rourke, K.I., Cheevers, W.P. Antigenic variation of neutralization sensitive epitopes of caprine arthritis-encephalitis lentivirus during persistent arthritis. *Journal of Virology* 62:3488-3492, 1988.
2. Cheevers, W.P., Stem, T.A., Knowles, D.P., McGuire, T.C. Precursor polypeptides of caprine arthritis-encephalitis lentivirus structural proteins. *Journal of General Virology* 69:675-681, 1988.
3. Archambault, D., East, N., Perk, K., Dahlberg, J.E. Development of an enzyme-linked immunosorbent assay for caprine arthritis-encephalitis virus. *Journal of Clinical Microbiology* 26:971-975, 1988.
4. Cheevers, W.P., Knowles, D.P., McGuire, T.C., Cunningham, D.R., Adams, D.S., Gorham, J.R. Chronic disease in goats orally infected with two isolates of the caprine arthritis-encephalitis lentivirus. *Laboratory Investigation* 58:510-517, 1988.
5. Knowles, D.P., Cheevers, W.P., McGuire, T.C., Stem, T.A., Gorham, J.R. Severity of arthritis is predicted by antibody response to gp135 in chronic infection with caprine arthritis-encephalitis virus. *Journal of Virology* 64:2396-2398, 1990.
6. Perk, K. The presence of virus particles in neural cells of caprine arthritis-encephalitis disease. *Research in Veterinary Science*, in press.
7. Chitlatur-Karpuz, E., Zimmer, A., Perk, K., Dahlberg, J.E. Characterization of structural polypeptides of lentiviruses. *Israeli Journal of Veterinary Medicine*, in press.

TABLE 1

Effect of rWRCo and rWR63 gp135 on serum neutralization of CAEV(Co)

Dilution	Percent TCID ₅₀ /ml neutralized					
	rWRCo		rWR63	rWRpSC11	DME	
	1	2	1	1	1	2
1:1	37.56	72.33	86.02	>99.96	>99.96	>99.85
1:2	94.43		99.96	>99.96		
1:4	97.21		>99.96			
1:5		97.85				
1:8	>99.96		>99.96			

Neutralization assays employed the virus titer reduction method using serum 89G38 from a goat infected for 1 year with CAEV(Co). The mean titer of CAEV(Co) incubated with four control sera from age-matched CAEV-negative goats was 7.32×10^4 TCID₅₀/ml for experiment 1 and 2.08×10^4 TCID₅₀/ml for experiment 2.

TABLE 2

Neutralization of CAEV(63) by sera from goats vaccinated with rWR63

Serum	4 weeks postvaccination		5 weeks postvaccination	
	TCID ₅₀ /0.1ml neutralized	% neutralization	TCID ₅₀ /0.1ml neutralized	% neutralization
89G04	0	0	4.33x10 ³	41.28
89G50	2.62x10 ³	41.95	9.50x10 ²	9.05
89G54	0	0	1.99x10 ³	18.94
89G59	0	0	4.19x10 ³	39.91
89G65	3.91x10 ³	62.52	7.68x10 ³	73.16
89G66	3.67x10 ²	5.86	6.87x10 ³	65.42

The mean titer of CAEV(63) incubated with four control sera from age-matched CAEV-negative goats was 6.26×10^3 TCID₅₀/0.1 ml for the 4 weeks postvaccination assays and 1.05×10^4 TCID₅₀/0.1 ml for the 5 weeks postvaccination assays.

TABLE 3

Neutralization of CAEV(Co) by sera from goats vaccinated with rWRCo

Serum	Percent neutralization (Initial titer = 8.36×10^4 TCID ₅₀ /ml)		
	20 weeks (pre 3rd vaccination)	22 weeks (2 weeks post 3rd vaccination)	25 weeks (5 weeks post 3rd vaccination)
89G25	37.10	66.16	32.98
89G26	9.30	66.87	51.20
89G65			63.71

89G65 serum is 5 weeks postvaccination with rWR63 (Table 2). Note that this rWR63-specific serum neutralizes CAEV(Co) as well as CAEV(63).

TABLE 4

ELISA detection of CAEV antigen in milk samples using monoclonal antibodies

Sample	Optical density
Normal goat milk	0.101
Normal cow milk	0.116
Normal goat milk whey	0.091
Normal cow milk whey	0.112
Infected goat milk #1	0.671
#2	0.453
#3	0.191
#4	0.391
CAEV 5 ng	0.276
8 ng	0.291
10 ng	0.517

TABLE 5
Serology of Israeli goats

Herd	Breed	Positive/total serums examined		
		ELISA	RIA	AGID
P.H.	Local	0/11	0/11	0/11
M.A.	Local	0/10	0/10	0/10
A.A.	Local	0/15	0/15	0/15
P.I.	Local	0/10	1/10	0/10
	Total	0/46	1/46	0/46
O.N.	Saanen	9/10	10/10	2/10
B.O.	Saanen	6/10	7/10	1/10
C.N.A	Saanen	10/10	10/10	2/10
A.G.	Saanen	8/10	9/10	1/10
C.N.B.	Saanen	10/10	10/10	3/10
R.G.	Saanen	8/10	8/10	1/10
L.M.	Saanen	9/10	8/10	3/10
N.S.	Saanen	7/10	7/10	2/10
M.S.	Saanen	8/10	8/10	3/10
G.O.	Saanen	8/12	ND	3/12
L.S.	Saanen	8/11	ND	3/11
O.R.	Saanen	10/14	ND	2/14
N.A.	Saanen	13/15	ND	5/15
	Total	114/142	77/90	31/142

TABLE 6
Serology of Israeli goats

Farm	Breed	Positive/total serums examined	% positive
14	Saanen	43/54	80
15	Saanen	40/57	70
16	Saanen	65/75	86
N-1	Local	0/42	0
N-2	Local	3/58	2

TABLE 7

Effect of Pasteurization on CAEV seroconversion

Group	Treatment	Positive/negative
1	Cow colostrum	1/20
2	Pasteurized goat milk	7/30
3	None	18/20

TABLE 8

Effect of Pasteurization on CAEV seroconversion

Farm	Treatment	Positive/total serums examined	Questionable
A1	Pasteurization	1/30	1
Da	"	1/32	2
M1	"	1/10	1
		Total	3
Be	None	9/19	7
K1	"	122/231	51
		Total	58

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6