Infection of Lactating Goats by Mammary Instillation of Cell-Borne Caprine Arthritis-Encephalitis Virus

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ABSTRACT

The possibility of transmission of caprine arthritis-encephalitis virus between lactating goats by the transfer of infected cells during milking has been examined experimentally. Four virus-free Saanen goats were infected, two in full lactation and two during the drying off period, by instillation of infected allogeneic cells into the milk canal of one-half of the udder. All four goats became infected, as shown by the isolation of virus from peripheral blood monocytes, seroconversion, and presence of typical lesions in the mammary gland. The virus was rapidly and constantly demonstrable in cells from the mammary secretions of the dry goats, but less regularly detected from milk cells from the lactating goats. Virus was more frequently isolated when milk SCC were elevated. Virus was present as often in secretions from the uninoculated half of the udder as the inoculated half. Dry goats seroconverted progressively, although antibody concentrations remained low; the lactating goats developed weak, positive Western blots in the late phase of lactation but only gave positive ELISA titers after drying off and artificial reinduction of lactation. At necropsy, lesions typical of the virus were observed in the mammary tissues of all four goats, and three had arthritis of the carpi.

(Key words: goats, caprine arthritis-encephalitis virus, milking, transmission)

INTRODUCTION

Caprine arthritis-encephalitis virus (CAEV), a lentivirus that is closely related to the visna-maedi virus of sheep, in goats causes persistent infections with long latent periods (12). Infection of goats with CAEV induces various lesions and has economic consequences arising from a shortened useful lifespan, loss of milk production, and mortality of kids born to infected dams (13). Viral infection is accompanied by an induration of the udders without individualizable nodules and hypertrophy of the retromammary lymph nodes (7, 9, 20).

We have previously shown (9) that the milk of infected goats contains cells that are capable of producing an infectious virus that may contribute to transmission to young kids and that viral expression was more easily demonstrated in cells immediately after parturition and artificial induction of lactation. A similar relationship between the stage of lactation and the ease of detection of a small ruminant lentivirus in milk cells has been demonstrated (16) in an ewe infected with visna-maedi virus; in that experiment, both natural parturition and a later artificial induction of lactation induced the increased production of virus-infected macrophages in the milk. Thus, production of infectious virus and, possibly, susceptibility of mammary tissues to infection may be influenced by physiological factors correlating with the stage of lactation.

A risk of infection of healthy herdmates by reflux of virus-producing cells in milk from an infected goat during mechanical milking appears to be likely. A preliminary study showed

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Abbreviation key: CAEV = caprine arthritis-encephalitis virus.
that visna-maedi virus, inoculated into the udder of ewes, induced specific histological lesions after 5 mo (18), and the authors concluded that the udder is a possible entry portal for virus. It would appear to be prudent herd management to milk healthy seronegative goats first to limit the dissemination of virus. In France, goats are dried off by gradually decreasing frequency of milking, and, although the susceptibility of the caprine udder to infections at this stage has not been studied, mammary infections of cows are more frequent in early dry period than in the rest of the reproductive cycle (15). It is generally recommended that abrupt drying off of cattle reduces the risk of mastitis.

In view of these observations from previous reports, which suggest that physiological factors correlated with lactation stage may influence the susceptibility of the udder to infection, we performed an experiment designed to test whether CAEV-infected cells could induce systemic infection when instilled into the teat canals of healthy goats and to examine the influence of the physiological status of the mammary gland on the development of the infection.

MATERIALS AND METHODS

Goats

Four Saanen goats (Table 1) were obtained from a CAEV-negative herd and had no detectable specific antibodies to the virus. Coculture of their milk cells with indicator cells detected no CAEV before inoculation. Two pregnant goats were inoculated during the early drying off period, 2 and 3 mo before parturition. Samples of blood and mammary secretions were withdrawn for serology, SCC, and viral evaluation at approximately 10-d intervals until parturition, and then weekly during the month following birth. Two other goats were inoculated during the 1st mo of lactation, and the presence of circulating antibodies, SCC, and the presence of virus in the milk cells were measured at regular intervals for 2 and 3 mo postinoculation, respectively. These goats were then dried off over 2 to 3 mo, and lactation

<table>
<thead>
<tr>
<th>Goat</th>
<th>Stage</th>
<th>Period1</th>
<th>Virology2</th>
<th>Western3 blot</th>
<th>ELISA4</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Infected teat</td>
<td>Control teat</td>
<td></td>
</tr>
<tr>
<td>83023</td>
<td>L1</td>
<td>A</td>
<td>70</td>
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<td></td>
<td>B</td>
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<td>2/2</td>
<td>2/2</td>
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<tr>
<td></td>
<td></td>
<td>C</td>
<td>120</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
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<td>C</td>
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<tr>
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<td>A</td>
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<td></td>
<td></td>
<td>C</td>
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<td>5/5</td>
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<td>A</td>
<td>34</td>
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<td></td>
<td></td>
<td>B</td>
<td>33</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>28</td>
<td>1/3</td>
<td>2/3</td>
</tr>
</tbody>
</table>

1Period A runs from time of viral inoculation until the first positive Western blot, period B runs from the end of period A until parturition or drying off, and period C runs from the end of period B until slaughter.

2The number of isolates giving positive viral cultures out of the number of cultures successfully performed during the indicated period.

3At least two specific viral bands present in Western blot, as described in the text.

4± = Marginal increase over baseline optical density, + = clearly increased optical density, as described in the text, and +, - = only the first of two tests was positive.
was artificially reinduced by treatment with estradiol-17β (.5 mg/kg per d) and progesterone (1.25 mg/kg per d) for 7 d, and then three injections of 25 mg of hydrocortisone (Roussel, Romainville, France) on d 18, 19, and 20.

One month after parturition or induction of lactation, and after clinical examination, the four goats were killed by intravenous injection of sodium pentobarbital (Dolethal, Lure, France). The presence of serum antibodies to CAEV and the presence of virus in blood and milk cells and in mammary tissue and retromammary lymph node explants were again determined. Histological sections of mammary tissue were prepared; the goats were carefully necropsied, and particular attention was given to the carpal joints.

Intramammary Inoculation

Cultured blood monocytes from a male goat that had been experimentally infected with the Cork strain of CAEV (3) were trypsinized and adjusted to a concentration of 5 x 10^5 cells/ml in Eagle's minimum essential medium (Gibco BRL, Cergy Pontoise, France). An inoculum of 2 ml of this suspension was introduced into one teat canal of each goat at the end of a manual milking session. Each goat received three such instillations into the same teat at 2-d intervals.

Presence of CAEV

Cells from defatted milk were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum (IBF, Villeneuve-la-Garenne, France) as described previously (16) and cocultured with caprine embryo fibroblasts in the same medium. The presence of virus was evaluated by counting syncytia with more than four nuclei after 3 wk of culture at 37°C. Virus in peripheral blood monocytes was evaluated by coculture as described by Guiguen et al. (5). Typical viral cytopathic effects were also sought in cells growing from explants of mammary tissue or lymph nodes (10).

Cytological Examination of Milk Cells

The suspension of washed cells used for culture was adjusted to 1 x 10^6 cells/ml in medium, and smears were prepared by cytocentrifugation. Cells were identified visually after staining with May-Grunwald Giemsa, and results were expressed as the number of cells per milliliter of secretion collected.

Microbiological Examination of Milk

The presence of bacteria was investigated at monthly intervals. A sample of milk (.025 ml) was spread on sheep blood agar, and bacterial species were identified after 24 and 48 h of incubation at 37°C (17).

Serological Testing

Antibodies to CAEV were evaluated by Western blotting using serum diluted 1:100 (1). Sera that reacted with both core protein (p27) and envelope glycoprotein (gp42) were considered to be positive. Antibody titers of the seropositive samples were estimated using an indirect ELISA technique (19). Samples were tested at 1:100 dilution in PBS containing .05% Tween 20, and results were measured as optical density units. All samples for a given goat were tested simultaneously on the same test plate, together with standard reference positive and negative samples, which determined the cutoff point for each plate.

Clinical and Pathological Examination

Before inoculation and again before slaughter, the goats were thoroughly examined, especially the udders and the carpi. Internal examination was performed at necropsy. Samples of mammary tissue and synovial membrane were prepared for histology, examined after staining with hematin-eosin-safran, and interpreted according to the criteria described previously (4, 18).

Statistical Tests

Results are expressed as means and standard deviation. Significance was evaluated using unpaired Student's t test.

RESULTS AND DISCUSSION

The introduction of CAEV-infected allogeneic macrophages into the milk canal of
TABLE 2. Relationship between milk SCC and the successful isolation of virus in goats that had been inoculated with caprine arthritis-encephalitis virus.

<table>
<thead>
<tr>
<th>Cell number (x 10^5)</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD (no.)</td>
</tr>
<tr>
<td>Total cells</td>
<td>8.68</td>
<td>.70</td>
</tr>
<tr>
<td>Macrophages</td>
<td>.56</td>
<td>.57</td>
</tr>
</tbody>
</table>

\(^1\)Number of measures.

one side of the udders of four Saanen goats at different stages of lactation induced generalized infection in all cases. Similar results for ewes that had been inoculated by visna-maedi virus were mentioned by van der Molen and Houwers (18), but experimental details were not given.

The virus was more regularly detected from secretions of the dry goats than from milk cells of goats in full lactation (Table 1). The percentage of macrophages was much greater in secretions from dry goats than in the milk of lactating goats (30% vs. 5%). The natural host cell for replication of CAEV is the macrophage (14), and secretions from dry goats not only contain more cells per volume than the milk of lactating goats (11), but most of the mononuclear cells in drying off secretions are macrophages (8). As shown in Table 2, the likelihood of positive viral isolation from milk cells of the lactating goats also correlated with the SCC and, particularly, the number of macrophages present. Except for one lactating half udder, none of the udders of the four goats showed an increase of the SCC from bacterial infection of the udder. The physiological condition of the udder after dry-off may also be more conducive to rapid viral infection (9). During the dry period, considerable changes in the structure of the mammary gland take place. The increased concentrations of lactogenic hormones during the last third of gestation induced an important development of the lobulo-alveolar tissue (2). The effect of these hormones on infected cells in the udder could increase the viral expression and consequently stimulate the development of the infection.

Virus quickly appeared in the uninoculated side of the udder, even though the two halves are anatomically well separated, and positive cultures from mammary biopsies confirmed the dissemination of infection (not shown). Spread from the inoculation site was also suggested by positive viral cultures of biopsy material from the retromammary lymph nodes (not shown). At a later date, virus was detected from circulating blood monocytes, confirming the eventual generalization of the infection. At slaughter, 3 to 5 mo postinoculation, all four goats had characteristic mammary histological lesions consisting of follicular lymphoid hyperplasia around lactiferous ducts, interstitial infiltration of mononuclear cells, and some fibrosis. Three goats had typical macroscopic and histological arthritic lesions of the carpal joints with a hyperplastic synovial membrane and subsynovial lymphoid cell infiltration.

Antibody to CAEV was detected by Western blotting using antigen from the same Cork strain of virus that had been used to infect the goats. Western blots were considered to be positive only if antibodies to both core antigen (p27) and to an envelope glycoprotein (gp42) were clearly present. All serum samples from each goat were tested simultaneously by ELISA, using known positive and negative samples to calibrate the cutoff point. Seroconversion was weak but progressive in the goats that were inoculated while dry, and positive reactions were first observed only at 33 to 40 d postinfection, even with these quite sensitive techniques (Table 1). The goats that were inoculated during full lactation developed positive Western blots only toward the end of lactation. The ELISA determinations remained negative throughout the lactation period, despite positive viral cultures and evidence of viral dissemination. One of these goats clearly seroconverted after drying off, a rest period, and then artificial reinduction of lactation; the other showed a single clearly positive result by ELISA (Table 1).

This delayed and unreliable seroconversion, using techniques more sensitive than the stan-
standard immunodiffusion assay, suggests that serological testing may not be adequate for surveillance of the spread of CAEV infection in controlled flocks. Virus could regularly be shown to be present in milk or mammary secretions at times before even the Western blot became positive for antibody. A similar insensitivity of serological methods was reported by Johnson et al. (6), who showed that only 25 to 30% of a series of goats were seropositive by ELISA and Western blot, but 65 to 70% were positive for virus by in situ hybridization or polymerase chain reaction. Unfortunately, these sensitive techniques are not practical for the routine identification of infected goats in farm herds.

Our results suggest that the reliance on agar gel diffusion serology for preventive detection of CAEV infection in France grossly understimates the number of infected animals and may explain the disappointing results of some attempts at prevention programs.

Our results show that intramammary inoculation of CAEV-infected allogeneic cells at any period in the milking cycle leads to generalized infection. Infected cells appeared rapidly in the milk of the infected goats, before the appearance of specific antibodies. During the drying off period, the number of infectious macrophages in the secretions rises in both infected and healthy goats, and the virus is more regularly detected from infected goats. Goats infected during the dry period showed more rapid viral and antibody responses than those infected during full lactation, which did not respond fully until they too entered drying off. This suggests an important contribution of the lactation stage on the immediate outcome of infection by CAEV.

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