

Susceptibility of Blood-Derived Monocytes and Macrophages to Caprine Arthritis-Encephalitis Virus

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Permissiveness of blood-derived caprine monocytes to infection by caprine arthritis-encephalitis virus increased during *in vitro* cultivation and differentiation into macrophages, as evidenced by immunofluorescence and release of extracellular infectious virus. The degree of cell susceptibility to virus infection varied among individual goats, independent of age or breed. Caprine arthritis-encephalitis virus infection of macrophages *in vitro* resulted in no alteration of five characteristic functional activities.

Caprine arthritis-encephalitis virus (CAEV) is a nononcogenic retrovirus of the subfamily Lentivirinae (6). The virus was initially isolated from synovial tissue of a naturally infected arthritic goat by Crawford et al. (8). The natural infection of goats or experimental infection of Caesarean-derived goats with CAEV results in a persistent infection manifested as acute leukoencephalomyelitis in young goats (7) and a proliferative synovitis and periartthritis in older animals (9). The induction of a progressive inflammatory arthritis by CAEV is particularly interesting, since it closely resembles the histopathology of rheumatoid arthritis in humans (1, 15).

The association of viruses with mononuclear phagocytic cells is generally thought to play a role in the pathogenesis of many chronic viral infections (21, 22). That mononuclear cells are associated with persistent virus infection in the CAEV syndrome has been suggested by studies demonstrating the successful isolation of CAEV from buffy coat cells of the blood and from macrophages in the synovial fluid of chronically infected goats (1; Klevjer-Anderson et al., submitted for publication). Since mononuclear phagocytic cells are an integral component of pathological lesions, the interaction of CAEV with these cells may play an important role in the development and maintenance of arthritic lesions. We have previously shown that macrophages are capable of hosting CAEV replication *in vitro* (17). Our present work describes the replication kinetics of CAEV in blood-derived monocytes as compared to macrophages, the effect of caprine age and breed on this comparison, and initial experiments to determine the effect of CAEV infection on basic macrophage functions.

A single virus isolate cloned three times by

terminal dilution in goat synovial membrane cells was prepared from the prototype strain of CAEV, 75-G63 (8), and was used throughout these experiments. This virus stock contained $10^{6.41}$ 50% tissue culture infective doses per ml, as determined by twofold terminal dilution on synovial membrane cultures by using cytopathic effect as an indicator of infection (18). Cells were obtained from goats of two different age groups, those less than 1 year (82G's) and those from 3 to 4 years of age (78G's), and of three different breeds (Alpine, Nubian, and Toggenburg). Cultures of caprine monocytes were prepared by using standard Ficoll-Hypaque gradient techniques for the isolation of mononuclear cells (4). Cells were plated in Falcon 24 well plates in 100% donor calf serum containing penicillin (100 U/ml) and streptomycin (100 μ g/ml). Nonadherent cells were removed 2 to 4 h later, and of the remaining cells, 75 to 85% were designated monocytes as determined by α -naphthal esterase and differential Wright staining (2, 17). After 4 to 5 days of *in vitro* cultivation, the adherent cells remaining were designated macrophages (90 to 95%). The *in vitro* maturation of blood monocytes to macrophages has been extensively documented (3, 14, 19).

The ability of CAEV to infect and replicate in freshly isolated blood monocytes was compared to similar infections of macrophage cultures derived from blood monocytes by differentiation *in vitro*. The results are summarized in Table 1 and Fig. 1. Regardless of the age or breed of the donor, infected macrophages expressed immunofluorescence (IF)-detectable CAEV antigens earlier and to a greater extent than monocytes (Table 1). This difference in CAEV expression was maintained regardless of whether the cells were transferred 2 to 4 h before infection at $1 \times$

10^5 to 3×10^5 cells per well (to correct for increased cell spreading and decreased cell numbers in macrophage cultures) or infected in the wells initially used for plating. Although the percentage of cells expressing CAEV antigen does increase in monocyte cultures during the initial 7 days postinfection, it is not clear whether this rise is due to a gradual increase in virus expression in monocytes or to secondary infection of the increasingly differentiated cells. Titers of infectious CAEV released into the culture supernatants were also higher in infected macrophage as compared with monocyte cultures (Fig. 1). Electron microscopic examination of CAEV-infected macrophages on day 5 postinfection demonstrated mature virus budding into extracellular and intracellular spaces. Relative differences between individual goats in the total CAEV 50% tissue culture infective dose per milliliter per 10^5 cells and percent IF-positive cells were also detected (Table 1 and Fig. 1). This individual heterogeneity may be particularly significant, since a correlation between infectious virus expression in the joint and lesion development has been found in experimentally infected goats (Klevjer-Anderson et al., submitted for publication). The heterogeneity in virus susceptibility does not appear to be age (e.g., 78G13 versus 78G78) or breed (e.g., 78G13 versus 82G44) related. The increased susceptibility of macrophages for CAEV replication may be partly associated with increased virus adsorption, as indicated by the retention of four to five times the amount of radioactively labeled CAEV by macrophage versus monocyte cultures (data not shown). This may involve an increase either in the number of specific virus receptors or in nonspecific adsorption and phagocytosis, characteristics which may be more frequently associated with the differentiated macrophage. In addition, 20 to 30% of the macrophages *in vitro* had larger diameters than did monocytes and thus may adsorb more CAEV per cell based on surface area alone.

Since macrophages are an integral component of joint lesions in arthritic goats *in vivo* (1) and are susceptible to CAEV, we examined the effects of CAEV infection on characteristic macrophage properties. The infection and subsequent alteration of functional properties of mononuclear phagocytes by other viruses have been reported previously (5, 16, 20). For example, avian oncogenic retroviruses readily infect avian macrophages, with a resulting decrease in phagocytosis, cell adherence, acid phosphatase, and expression of F_c receptors (12). Five specific macrophage functions were examined after CAEV infection. All tests were performed on cells 4 to 7 days postinfection and compared with mock-infected cells from the same cell

TABLE 1. Expression of CAEV antigens in caprine monocytes and macrophages^a

Goat no. ^b and breed	Cell type	% IF-positive cells after the following no. of days postinfection ^c :		
		3	5	7
78G13 (Alpine)	Monocyte	<1	28.7	46.5
	Macrophage	30.8	55.0	74.6
78G78 (Nubian)	Monocyte	<1	8.7	19.4
	Macrophage	14.4	21.2	53.0
82G65 (Toggenburg)	Monocyte	<1	10.4	14.2
	Macrophage	10.1	33.1	41.4
82G66 (Toggenburg)	Monocyte	<1	10.3	27.3
	Macrophage	14.6	26.9	45.7

^a Cultures were washed once with Hanks balanced salt solution without Ca^{2+} and Mg^{2+} and infected at a multiplicity of infection of 4 to 6 with adsorption at 37°C for 90 min.

^b Goat number includes the year of birth (1978 or 1982) and the animal identification number (e.g., G13). No IF data were available for 82G44 (Alpine).

^c Cells were removed with 1% lidocaine in Hanks balanced salt solution without Ca^{2+} and Mg^{2+} , spun onto slides, fixed in acetone at -70°C for 1 h, and then stained by the direct IF technique with fluorescein isothiocyanate-labeled goat antiserum (1).

preparation. None of the five basic functional characteristics examined, including F_c and complement receptors, phagocytosis of latex particles, and acid phosphatase and *N*-acetyl- β -D-glucosaminidase activity, was significantly altered by short-term virus infection (data not shown). Long-term infections were not practical due to the difficulty in maintaining caprine macrophages *in vitro* for extended periods without substantial reductions in cell number (especially after CAEV infection). However, the lack of detectable alterations in macrophage function by CAEV cannot be considered definitive yet, since in some virus systems (i.e., infectious bovine rhinotracheitis) immunological functions of macrophages are altered before other functional changes become apparent (13). The study of CAEV-macrophage interactions should be pursued further because such an interaction could contribute to lesion development within the joint in several ways, including altering their immunological functions and stimulating secretion of inflammatory mediators (11).

In this study of CAEV-cellular interactions, we found that as cultures of monocytes became more differentiated, there was a concomitant increase in permissiveness for CAEV replication. Additional examples of virus-macrophage interactions exist which indicate similar viral pathogenesis, including herpes simplex type 1, which replicates more efficiently in well-differentiated macrophages than in blood monocytes

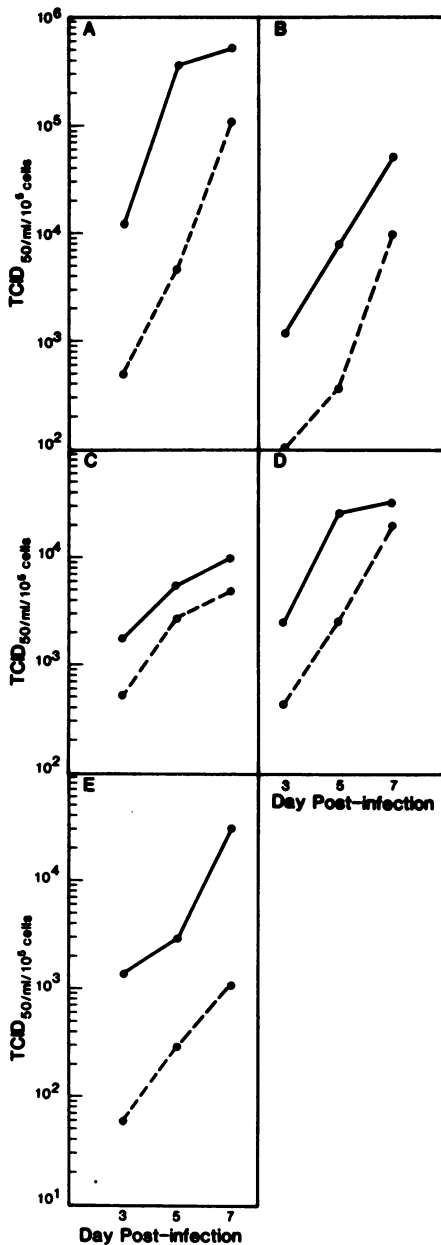


FIG. 1. Infectious CAEV released by infected caprine monocyte and macrophage cultures. (A) 78G13; (B) 78G78; (C) 82G65; (D) 82G66; (E) 82G44. Macrophage cultures are represented by a solid line, and monocyte cultures are represented by a broken line. Cultures were infected as described in footnote *a* of Table 1. The medium was changed 24 h before harvest of culture supernatants for virus titration. Supernatants were portioned and frozen at -70°C until titer determination. Results represent the means of duplicate experiments. TCID_{50} , 50% tissue culture infective dose.

(10, 23). Thus, in the CAEV syndrome, our data suggest that monocytes migrating from the circulation into the infected joint would be susceptible to virus infection and thereby may aid in perpetuating instead of eliminating the infection. Also, low or latent virus expression by blood monocytes could aid in the distribution of CAEV to distant sites, with a subsequent burst of virus production after migration and differentiation in various tissues. In light of the variability in virus production by macrophages from individual animals, and since macrophages are prominent in the synovial fluid and synovial tissue of arthritic animals, further investigation of virus-macrophage interactions is warranted to determine more completely their role in the pathogenesis of caprine arthritis.

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