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Viral expression and leukocyte adhesion after in vitro infection of goat mammary gland cells with caprine arthritis-encephalitis virus

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Abstract

A characteristic lesion in goats infected by the lentivirus CAEV is mastitis with lymphoid hyperplasia. In order to investigate the mechanism of lesion formation, cultures highly enriched in microvascular endothelial cells, mature and immature luminal epithelial cells, fibroblasts and myoepithelial cells were established from goat mammary gland biopsies. Their susceptibility to in vitro infection with two distinct types of CAEV was investigated by PCR, antigen expression and cytopathy. The capacity of infected mammary gland cells to bind uninfected caprine leukocytes was determined by flow cytometry. All cell types tested were susceptible to CAEV infection in vitro, with different levels of sensitivity according to cell phenotype. Our results suggest that the limited extent of natural infection of mammary gland cells reflects a protective local immune response, and that the myoepithelial cell could act as a reservoir cell. After infection, the mature luminal cell acquires the capacity to bind leukocytes in vitro, which could indicate a facilitation of cellular interactions. The distinct reactions of the different cell types to CAEV infection may be correlated with events leading to progressive lesion development during the natural infection.
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1. Introduction

The constitution of a tissue lesion consequent upon viral infection involves disruption of the relationships between the different cell types constituting the infected tissue. An understanding of these processes, together with the local immune reactions, is necessary for envisaging effective therapy. One consequence of the infection of goats by the lentivirus caprine arthritis-encephalitis virus (CAEV) is the development of mastitis, with consequent viral contamination of the milk and infection of suckling kids (Clements

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and Zink, 1996). CAEV, like other lentiviruses, productively infects cells of the monocyte-macrophage lineage, which distribute the viral infection throughout the body. This lentivirus does not infect lymphocytes, but has been reported to infect several types of endothelial and epithelial cells in vitro (Le Jan et al., 2000; Lerondelle et al., 1999), although the importance of this infection in vivo is still unclear. The natural course of CAEV infection alternates periods of reduced viral expression with bursts of circulation of productively infected monocytes, particularly under the hormonal influence of parturition. Similar stimulation of a quiescent lentiviral infection can be seen in HIV-1 patients on immune stimulation (Goletti et al., 1996), or in Maedi-Visna virus infected sheep at lambing (Pépin et al., 1998). The presence of circulating infected cells may modify the dynamics of leukocyte migration through the vascular endothelium (Ghorpade et al., 2001), but little is known of the events within the target tissues leading to the formation of a lesion. It is becoming clear that lentiviruses, including CAEV, can infect non-leukocytic cells in vivo (Sanna et al., 1999; Carrozza et al., 2003; Chi et al., 2000), including microglial cells, astrocytes and oligodendrocytes, types I and II pneumocytes, and mammary fibroblasts, epithelial and endothelial cells. These susceptible cells in the target tissues might not only provide a reservoir of virus, but also constitute a mechanism for lesion formation through alterations in their interactions with their neighbors and presentation of antigens for a local immune reaction. We have used the mammary glands of non-infected goats to study the susceptibility of the different tissue types to infection by two distinct isolates of CAEV in vitro, and to study the phenotypic consequences of infection, particularly the modification of leukocyte adhesion. In natural infections, virus expression is initiated at parturition, and continues during the lactation period, favoring infection of progeny. Viral expression regresses with mammary involution and re-activates at subsequent mammogenesis, so that peak viral production corresponds to the most favorable time for transmission (Clements and Zink, 1996). We have therefore studied the capacity of mammary fibroblasts, macro- and micro-vascular endothelial cells, mature and immature luminal epithelial cells and myoepithelial cells to support CAEV infection and production in vitro, and any consequent modification in capacity for leukocyte binding.

2. Materials and methods

2.1. Animals

Tissue and blood samples were taken from four Saanen goats from breeding stocks with a sustained history of negativity for CAEV infection by both ELISA serology and PCR testing of blood leukocytes (Morin et al., 2003). Housing and experimental procedures were approved by the ethics committee of the Lyon National Veterinary School. Two animals provided mammary gland biopsies, at the lactating and involution stages, respectively, after general anesthesia (Dolethal, i.v. 200 mg/kg body weight; Vetoquinol, Lure, France) and subsequent euthanasia. A third goat was sacrificed and the aorta dissected out at autopsy. Blood for the preparation of peripheral blood mononuclear cells (PBMC) was obtained from a fourth animal by jugular venupuncture onto heparin.

2.2. Cell cultures

Mature luminal epithelial cells, myoepithelial cells, fibroblasts and microvascular endothelial cells were prepared from the lactating goat biopsy. For the mature epithelial cells, four fragments of ca. 0.25 cm^3 were digested in 15 mL basal medium supplemented with hyaluronidase type III (Sigma, Saint Quentin Fallavier, France; 200 IU mL^{-1}) and collagenase type III (Sigma; 200 IU mL^{-1}) in 25 mL conical tubes under intermittent agitation for 1.5 h at 37 °C. Detached cells were filtered through gauze, recovered by centrifugation (430 g, 10 min, 4 °C) and cultured in collagen-coated flasks (Biocoat collagen I cellware; Beckton-Dickinson, Grenoble, France) in basal medium containing 10% foetal calf serum (FCS, Invitrogen, Cergy Pontoise, France), 5 mg mL^{-1} bovine pancreatic insulin (Sigma) and 10 ng mL^{-1} epidermal growth factor (Sigma). Basal culture medium consisted of RPMI (Invitrogen) supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 2.5 mg mL^{-1} amphotericin B (Invitrogen). These cells were always studied at the second culture

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