Caprine Arthritis-Encephalitis Virus Infection of Caprine Monocytes

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SUMMARY

Monocyte-enriched cultures of goat peripheral blood leukocytes were exposed to caprine arthritis-encephalitis virus (CAEV) at an m.o.i. of 8 TCID₅₀ per cell as measured in goat synovial membrane cell cultures. At the time of infection, 90% of the adherent cells displayed characteristic macrophage markers of phagocytic activity and cytoplasmic non-specific esterase. A productive infection was established, with extracellular infectious virus titres reaching a maximum of $10^{5.2}$ TCID₅₀ per ml at day 4 post-infection. Synthesis of CAEV antigens was detected by direct immunofluorescence and it was shown that 85% of the adherent cells contained virus antigen by day 6 post-infection. A cytopathic effect, characterized by giant cell formation and cell death, developed concomitantly with increased virus replication.

Caprine arthritis-encephalitis (CAE) syndrome is a naturally occurring persistent virus infection of goats (Crawford et al., 1980a). This disease syndrome is most often manifested as (i) acute leuko-encephalomyelitis occurring with low incidence primarily in young goats (Cork et al., 1974) and (ii) chronic proliferative synovitis and periarthritis in a high percentage of adult animals (Crawford et al., 1980b). The aetiological agent of this disease complex, designated caprine arthritis-encephalitis virus (CAEV), has been classified as a retrovirus based on characteristic physical and biochemical features (Crawford et al., 1980a; Cheevers et al., 1981). In vivo, CAEV demonstrates a tropism for synovial membrane cells, but can also be recovered from many other tissues including brain, lung, kidney and spleen (Adams et al., 1980; P. Klevjer-Anderson, unpublished results). The CAE syndrome represents an interesting chronic disease in that the joint lesion which develops as a result of virus infection closely resembles rheumatoid arthritis in man. Thus, investigation of this virus–host relationship presents a unique opportunity for arthritis research.

The association of viruses with mononuclear phagocytic cells is generally thought to play a role in the pathogenesis of many chronic virus infections (Mims, 1974). This role seems to be related to the ability of these viruses to persist and replicate in macrophages. Furthermore, virus infection may alter the ability of macrophages to cooperate with lymphoid cells in the induction of an immune response. Little is known concerning the effect of CAEV on goat macrophages. CAEV can be isolated from the buffy coat fraction of blood and from monocytes in the synovial fluid of persistently infected goats (Adams et al., 1980; P. Klevjer-Anderson, unpublished results). In the experiments reported here, we examined the ability of peripheral blood mononuclear cells to support CAEV replication in vitro.

Monocytes were isolated by centrifugation of peripheral blood in Ficoll-Hypaque gradients as described by Banks & Greenlee (1981). The mononuclear cells were seeded into Linbro 24-well tissue culture plates in 100% donor calf serum (DCS). After 24 h the cultures were washed to remove non-adherent cells and fresh media added to the adherent cells. At the time of virus infection, the adherent cell population consisted of greater than 90% monocytes as judged by their ability to phagocytose carbon particles, the presence of cytoplasmic non-specific esterase and resistance to detachment by trypsin (Banks & Greenlee, 1981). The results described below were derived from CAEV infection of monocytes from a single goat.
Fig. 1. Replication of CAEV in goat peripheral blood monocyte cultures. At day 5 post-plating, replicate cultures were inoculated with CAEV at an m.o.i. of 8 TCID₅₀ per cell. ▲—▲, Extracellular virus, TCID₅₀ per ml per 24 h as titred by twofold terminal dilution on synovial membrane cells using c.p.e. as an endpoint. ●—●, Percentage of IF-positive cells. Monocytes were removed from culture by treatment with a 1:100 dilution of lidocaine (Hu & Muscoplat, 1980) for 30 min at 37 °C with vigorous pipetting. The cells were then centrifuged on to slides at 30000 cells/slide using a Shandon-Southern Cytospin and stained with fluorescein isothiocyanate-conjugated goat anti-CAEV. The antiserum was derived from a goat hyperimmunized with purified CAEV and detects principally CAEV p28. Cytopathic effect was scored from 1+ to 4+ daily depending upon the number and size of giant cells and the extent of cell death.

Fig. 2. CAEV infection of goat peripheral blood monocytes. Photographs of Giemsa-stained monolayers were taken at day 4 post-infection. (a) Control culture (mock-infected) showing typical macrophage morphology. (b) A culture productively infected with CAEV showing numerous giant cells and widespread cell destruction (c.p.e. 3+). Bar marker represents 200 μm for both (a) and (b).

However, monocytes from four other goats were also susceptible to CAEV infection (data not shown). All goats were 1 to 1.5 years of age and of the Toggenberg or Saanen breed.

On day 5 post-seeding, monocyte cultures were infected with cloned CAEV (prototype strain 75–G63) at an input multiplicity of 8 TCID₅₀ per cell. Cultures were pretreated with 4 μg/ml polybrene in RPMI 1640 for 1 h, then virus was allowed to adsorb at 37 °C for 2 h. The CAEV inoculum was removed, the cultures washed twice and fed 100% DCS plus 100 units/ml penicillin and 100 μg/ml streptomycin. The strain of CAEV used in these experiments was originally isolated from a goat with spontaneous arthritis (Crawford et al., 1980a) and is routinely propagated and titred in goat synovial membrane cells in vitro.
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(Klevjer-Anderson & Cheevers, 1981). On successive days post-infection, replicate cultures were monitored for the production of extracellular infectious virus, the development of virus antigen detectable by direct immunofluorescence (IF) and cytopathic effect (c.p.e.).

Fig. 1 demonstrates the kinetics of CAEV replication in goat peripheral blood monocytes. A productive infection was established with a maximum titre of $10^{5.2}$ TCID$_{50}$ per ml by day 4 post-infection. By day 5 post-infection the CAEV titre began to decrease due to a significant reduction in the number of adherent cells; virus-induced c.p.e. developed concomitantly with virus replication (Fig. 1). The c.p.e. was manifested as increased giant cell formation and cell death (Fig. 2).

The proportion of adherent cells showing evidence of CAEV infection was detected using a direct IF assay (Klevjer-Anderson & Cheevers, 1981). At 24 h a low percentage of cells was IF-positive (3 to 5%), but this percentage rose rapidly thereafter, in concert with virus replication, reaching a maximum of 85% by day 6 post-infection (Fig. 1). The inability to detect virus antigen in 100% of cells in infected cultures may have been due to (i) a heterogeneous cell population with regard to CAEV susceptibility, (ii) insensitivity of IF assay for low levels of virus antigen and/or (iii) the culture time being too short to allow all cells to develop detectable infection. Nevertheless, a large majority of the cells did contain virus antigen and very few morphologically unaltered cells remained by day 6 post-infection.

We have shown that CAEV is able to establish a productive infection of caprine peripheral blood monocytes in vitro involving the majority of adherent cells. The association of CAEV with monocytes also characterizes the disease in vivo. For instance, CAEV can be recovered from the buffy coat of persistently infected animals by co-cultivation with synovial membrane cells in vitro (P. Klevjer-Anderson & D. S. Adams, unpublished results). Early CAEV replication in the synovium after intra-articular injection induces an influx of predominantly monocytes into the joint space. Infectious virus can be recovered from this synovial fluid cell population (P. Klevjer-Anderson, unpublished results) and immunofluorescent and histochemical staining demonstrate CAEV antigens present in the macrophage-like cells (Adams et al., 1980).

Apart from being a means of virus persistence and distribution to distant sites, the infection of macrophages by CAEV could play a significant role in the pathogenesis of CAE. Firstly, virus infection of macrophages could interfere with one or more of the complex interactions these cells have with other components of the immune system. Indeed, neutralizing antibody has not been detected in goats infected for up to 1.5 years with CAEV or hyperimmunized against purified CAEV (P. Klevjer-Anderson & T. C. McGuire, unpublished results). While many other explanations could account for the lack of neutralizing antibody, this is consistent with macrophage dysfunction. Secondly, CAEV infection of macrophages may render them less active in the phagocytic clearance of phlogogenic materials. This could have a significant effect, especially in the joint space, resulting in a build up of materials normally phagocytized by synovial fluid monocytes and the synovial membrane type A (macrophage-like) cells lining the joint cavity (Barrett et al., 1977). Moreover, CAEV infection of macrophages could induce the release of active substances that contribute to the pathology of the disease (Adams et al., 1980; Marmion & MacKay, 1977).

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