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A study of the process of apoptosis in animals infected with the contagious ecthyma virus

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Abstract

Contagious ecthyma virus (CEV) is a disease caused by a parapoxvirus, also is a potent genetic carrier with the capacity for regulating apoptosis in the cells of infected skin, a mechanism that serves for evading the immune response of the host. It has been suggested that the virus may remain in the skin and be able to cause repeated infections in the same flock.

The effect of infection as well as the presence of contagious ecthyma virus was evaluated in terms of lesions and apoptosis in the skin of animals, infected both naturally and experimentally.

Samples used were obtained from a naturally infected sheep, 5 goats inoculated with CEV and a negative control. Samples obtained were longitudinally sectioned and processed using photon and electron microscopy, and embedded in paraffin and araldite. Samples embedded in paraffin were sectioned in 5 μm of thickness and dyed with orange eosin–hematoxilin G and Gomori's trichrom stain, apoptosis was demonstrated by the TUNEL assay, the viral antigen was revealed using polyclonal antibodies, and the presence of lymphocytes CD4+ and CD8+, with monoclonal antibodies. The samples processed in resin were cut to obtain semi-fine sections and dyed with toluidine blue-borax, and the ultra-fine sections were impregnated with lead citrate and uranyl acetate.

Observations were similar in both, the natural infected animal and the experimental group. Infiltration was observed as well as images suggestive of a process of apoptosis. The TUNEL assay demonstrated that the number of epithelial cells undergoing apoptosis diminished during the process and increased among defense cells, until they almost disappeared at the beginning of healing. Cells undergoing apoptosis were located near the sebaceous glands and pilose follicles. The infiltrated lymphocytes gradually diminished. The viral antigen was observed in cells with morphology suggestive of apoptosis, located in sebaceous

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glands and pilose follicles. Using electron microscopy, cells with morphology compatible with that of lymphocytes were observed to be undergoing apoptosis, but there was little evidence of viral particles.

Keywords: Apoptosis; Contagious ecthyma; TUNEL assay; CD4+; CD8+

1. Introduction

Contagious ecthyma is a disease caused by a particular parapoxvirus, which is characteristic during its severest phase produce lesions in progression from papules to pustules and then to thick scabs or crust, the pustulating lesions affect the mucocutaneous borders and mucous membranes of sheep and goats. The contagious ecthyma virus (CEV) causes a debilitating disease of high morbidity 80%, and low mortality 5–10%, which occasionally affects humans (Fields et al., 1996; Haig and Mercer, 1998; Tórtora et al., 1998; Mercer and Haig, 1999; Regenmortel van et al., 2000).

Young animals are particularly susceptible, suffering from lesions which commonly appear around the mouth and nostrils. The lesions may reduce their ability to feed themselves, to suckle and graze, and secondary infections may also appear along with bacteria, fungi or insect larvae (Haig et al., 1999). Ecthyma may become a serious problem, as it can provoke mortality in animals under stressful conditions and immune suppression among overcrowded sheep or animals transported over long distances (Haig and Fleming, 1999).

Certain aspects of the disease have still not been clarified, the virus can infect sheep and goats repeatedly, in spite of the fact that the host apparently presents a vigorous immune response and inflammation (Haig et al., 1996, 1997a,b). Another aspect which remains to be clarified is that among animals with skin infection, there is no evidence of systemic infection.

The virus is found in the material originating from scabs and it has been suggested that outbreaks occur because of contact with infectious viruses in the environment, or it is also possible that the virus maintains itself in the flock at sub-clinical levels, causing periodical undetectable damage to the skin, although this theory has not been rigorously explored (Haig and Mercer, 1997). There is however evidence of viral transmission between clinically healthy sheep and sheep that have not had contact with the virus (Nettleton et al., 1996).

The immune response against infection by CEV in skin and local lymph nodes, presents the characteristics of an antiviral reaction including CD4+, CD8+ cells, cytotoxic T lymphocytes, interferons, antibodies and other complementary components (Haig et al., 1998; Deane et al., 2000). Viral antigens have been identified which regulate the immunity of the host (Gooding, 1992), as well as genes which are related to virulence such as: a gene homolog for the vascular endothelial growth factor (VEGF) (Lyttle et al., 1994), a homolog of sheep interleukin-10 (IL-10) (Fleming et al., 1997) and a gene resistant to interferon (E3L) which is a homolog of the vaccinia virus gene (Chang et al., 1992).

Viral activity inhibits the granulocyte-macrophage colony-stimulating factor which may be responsible for temporary viral elusion, thus allowing replication in cells (Alcami and Smith, 1995; Haig et al., 1998; Alcami and Koszinowski, 2000; Deane et al., 2000). Among viral virulence mechanisms, the modulation of the apoptotic process and the genetic transmission of cells infected by the poxvirus may be considered (Haig et al., 1998; Turner and Moyer, 1998).

Apoptosis consists of the programmed death of cells, which permits the selective and rapid elimination of proliferating cell populations, a process which is observed during embryonic development, morphogenesis and metamorphosis, as well as in the atrophy of endocrine tissue, in the normal change and transformation of tissues, in the removal of undesirable cells during recovery from inflammation and during neoplastic regression (Aderem and Underhill, 1999; Cotter, 1990). Programmed cell death also plays a decisive role in the development and maturation of T lymphocytes (Rubin and Kretz-Rommel, 1999), the elimination of lymphocytes which recognize the self in the thymus gland, it preserves peripheral homeostasis and promotes tolerance towards T cells