An essential role for γ-herpesvirus latency-associated nuclear antigen homolog in an acute lymphoproliferative disease of cattle

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**AUTHOR SUMMARY**

The herpesvirus family encompasses a number of DNA viruses known to infect humans and animals. During infection, the herpesviruses often adapt to their host species, a phenomenon known as cospeciation (1). In few instances, however, the virus is transmitted between species and infects a nonadapted host, which can result in severe pathologies. *Alcelaphine herpes-virus 1* (AlHV-1) belongs to the γ-herpesvirus subfamily and naturally infects the wildebeest (Connochaetes spp.) without causing any clinical signs or lesions. However, when transmitted to other susceptible species such as cattle, this virus induces a severe acute and lethal disease known as malignant catarrhal fever. The mechanisms of this infection remain unclear.

In this study, we describe evidence that the virus must be dormant in the host during a period of latent viral persistence to induce malignant catarrhal fever in susceptible species such as cattle or an experimental rabbit model.

Wildebeests, also called gnus, belong to the subfamily *Alcelaphinae*, family Bovidae. These African antelopes are known for their remarkable annual migrations through the savannah in the Serengeti and Maasai Mara national reserves. Although this natural wonder attracts many tourists in Kenya and Tanzania, the livelihood of local farmers, such as the Maasai people, is directly endangered by the proximity of wildebeest herds. The Maasais are warriors and nomadic pastoralists whose social and economic wealth depends on livestock herding. Unfortunately, the wildebeest population carries the γ-herpesvirus AlHV-1, which proves deadly when transmitted to cattle and other multiple ruminant species. Nearly the entire wildebeest population is infected with AlHV-1, but these animals do not display clinical signs or lesions. The AlHV-1 virus is transmitted by ocular and nasal secretions. Whereas adults are not usually infectious, young wildebeest calves excrete the virus during the calving season and during the first 3–4 mo of life. Infected wildebeests carry the virus for life. These periods of migration are highly problematic for the Maasai cattle herds that share the grazing areas with infectious wildebeests (2).

Malignant catarrhal fever is an acute, lethal, sporadic, and pancytotoxic lymphoproliferative disease. The mechanisms of pathogenesis remain, however, unclear, making it difficult to develop vaccines and/or therapeutic strategies. In this study, we unraveled important mechanisms by which AlHV-1 induces malignant catarrhal fever in susceptible species. We experimentally induced malignant catarrhal fever in calves by AlHV-1 infection and performed a high-density tiling array of the entire AlHV-1 genome to show that only 10% of the virus sequence is expressed in the lymph nodes of sick animals. We did not observe significant expression of genes known to be essential for virus productive replication; however, a protein known as ORF73, which is essential for genome maintenance of γ-herpesviruses during latency, was highly expressed. Thus, we concluded that AlHV-1 infection is predominantly nonreplicative during the pathogenesis of malignant catarrhal fever. Our results confirm previous observations showing that no detectable infectious particles could be found in malignant catarrhal fever lesions (3). We also used a whole-genome approach to show that the gene expression signature in cells from infected animals was profoundly modified, with increased expression of numerous genes involved in the immune response, including cytotoxic granules, genes involved in cell division, and proinflammatory cytokines. Disruption of the host gene expression profile might reflect the proliferation of activated CD8+ T cells in the tissues of animals during the development of malignant catarrhal fever. The main lesions observed in symptomatic animals seem to be formed from the infiltration of lymphocytic cells in the perivascular spaces of...
These infiltrates likely reflect the expansion of uninfected or infected cells. Our analyses show that the AlHV-1 genome is maintained as circular episomes in vivo, a signature of \( \gamma \)-herpesvirus latency. Additional studies revealed that most of the cells constituting these infiltrates are infected by AlHV-1. These results were important, because they strongly suggested that malignant catarrhal fever could represent a virus-induced peripheral T-cell lymphoma caused by the proliferation of latently infected cells.

To address the role of latency in vivo, we generated virus strains that were impaired for ORF73 expression. These viruses should, therefore, not be able to establish latency in infected animals. Although the produced recombinant viruses could replicate in vitro and in vivo at the first days postinfection, they were not able to induce malignant catarrhal fever, and they were not able to persist after long-term in vivo infection. Although \( \gamma \)-herpesviruses require in vivo expression of ORF73 to persist in their respective natural hosts, we unveil evidence that malignant catarrhal fever, an acute and lethal lymphoproliferative disease, is induced by ORF73-mediated persistence and latency in susceptible species (Fig. P1).

Finally, we tested the potential of the ORF73-deficient recombinant viruses as candidate vaccine against malignant catarrhal fever using a recombinant attenuated virus that is unable to persist in vivo and represents a great hope for local pastoralists in East-Africa.

The AlHV-1 virus provides a useful example of coevolution between a virus and its host, because this herpesvirus is so adapted to its natural host that it is able to persist without causing lesions. By contrast, AlHV-1 transmission to other ruminant species causes malignant catarrhal fever, which ultimately kills the infected animal. The relationship between AlHV-1 and the wildebeest might be interpreted as beneficial for the virus alone. However, we propose that this relationship represents an evolutionary symbiotic adaptation for both species. According to this hypothesis, the adaptation of AlHV-1 to its host would allow viral persistence, and the wildebeest would benefit from the infection of competing species by gaining access to more food and/or providing weakened animals for large predators during the wildebeest calving period.

The findings presented in this study enhance our understanding of the pathogenesis of malignant catarrhal fever and represent an important step in the global appreciation for the unique evolutionary relationship between a virus and its many hosts.