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Research paper

The importance of FMDV localisation in lymphoid tissue

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ABSTRACT

Foot-and-mouth disease virus, a highly contagious pathogen that can cause lameness, low weight and decreased milk production, is a scourge of agricultural livestock around the world. Although the acute phase of infection is rarely fatal, infection may persist in animals that have apparently recovered, creating a viral reservoir that some fear could contribute to the spread of disease. We have used an array of molecular techniques to search for traces of virus in tissues from the mouths and throats of infected cattle. In a carefully controlled study, we have found evidence of intact, non-replicating virus particles trapped by follicular dendritic cells within the germinal centres of lymph nodes. Strikingly, virus was present for up to 38 days post infection, even though it was undetectable in surrounding tissues. The retention of intact virus within germinal centres is likely to have a role in stimulating the long lasting immune response that is characteristic of viral infections. Our data suggests that this capture may also be responsible for preserving intact viruses capable of infecting susceptible cells as they come into contact with germinal centres.

African buffalo (*Syncerus caffer*) are typically infected with all three South African Territories types of FMDV by 2 years of age and these viruses can be transmitted to farmed livestock. Buffalo harbour persistent virus in greater amounts and for longer periods than cattle and thus provided us with further opportunities to define the sites of viral localisation.

Foot-and-mouth disease (FMD) is a highly contagious, acute viral disease of cloven-hoofed, domesticated and wild animals, characterized by fever, loss of appetite, depression, lameness and the appearance of vesicles on the feet and in, or around, the mouth. The virus can spread extremely rapidly, has the potential to cause enormous losses and is the single most important constraint to international trade in livestock and animal products. Spread of the virus can be controlled by early detection of new cases, slaughtering animals on affected farms, restricting the movement of animals and inanimate objects likely to have become contaminated with the virus and vaccination of susceptible hosts.

The FMD virus (FMDV) particle consists of a single-layered protein shell (capsid) surrounding the single-stranded RNA genome. The genome is translated into an open reading frame polyprotein that is cleaved by virally encoded proteinases to produce mature viral proteins. Four of these proteins, termed VP1 to VP4, form the viral capsid (Grubman et al., 2008). FMDV is categorised according to the degree of cross-neutralisation into seven serotypes, named A, O, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. Animals that have recovered from infection with one serotype are normally protected against that serotype but remain fully susceptible to infection by any of the others. However, within each serotype group there are a number of strains which can differ genetically by

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up to 15% based upon VP1 gene analysis alone (Knowles and Samuel, 2003). This variation makes vaccine development and contingency strategy planning difficult as vaccine derived immunity is often strain specific, providing little intra-strain protective immunity.

In cattle, the viraemic phase of FMD lasts for approximately 3–5 days and virus is normally cleared from the peripheral epithelial sites associated with FMD vesicles within 2 weeks (Salt, 2004; Zhang and Alexandersen, 2004). However, FMDV can cause a prolonged, asymptomatic persistent infection in ruminants, leading to the carrier state; the period after 28 days post infection in which infectious FMDV may be detected on at least one of multiple oesophageal–pharyngeal samples (Sutmoller and Gaggero, 1965). Although the infectivity of persistently infected domestic ruminants has never been demonstrated under experimental conditions, they are perceived as a major threat and dictate trade policies and FMD control strategies.

Clinical signs of FMD are believed to be rare in free-living African buffalo (*Syncerus caffer*) populations (Vosloo et al., 2007; Thomson et al., 1992), although there is evidence in the literature that buffalo infected under experimental conditions show similar clinical signs as those reported for domestic ruminants (Vosloo et al., 2007; Dawe et al., 1994; Gainaru et al., 1986; Young et al., 1972). In southern Africa the buffalo act as long-term maintenance hosts for the three SAT serotypes of FMDV. Virus has been successfully isolated from captive buffalo held in isolation for 5 years and from a small free-living isolated population for 24 years (Condy et al., 1985). Persistently infected buffalo can maintain more than one type of SAT virus (Anderson et al., 1979; Hedger, 1972) and buffalo herds in the Kruger National Park, South Africa, usually harbour all three SAT types simultaneously (Hedger, 1972; Condy et al., 1969). Genetic and antigenic analysis of viruses isolated from individual animals indicates that persistently infected buffalo are a significant source of viral diversity (Vosloo et al., 1996). The ability of persistently infected buffalo to constantly generate variants by an unascertained mechanism and the ability of carrier buffalo herds to infect naïve animals, and domestic livestock in particular, complicates control of the disease through immunization (Vosloo et al., 1996, 2002; Dawe et al., 1994; Bengis et al., 1986). Although a number of studies have investigated the localisation of FMDV genome and viral proteins in tissues of domestic ruminants (Juleff et al., 2008; Alexandersen et al., 2002), there are no reports on the sites of virus localisation or replication in tissues of African buffalo after the acute stages of infection.

Lymphoid follicles are structures responsible for the selection and maturation of B cells during a humoral immune response. Secondary follicles develop discrete functional regions, designated the dark zone and light zone, which constitute a germinal centre. Follicular dendritic cells (FDCs) (Chen et al., 1978) are specialised, non-endocytic, immune accessory cells found in the follicles that play a key structural and functional role in follicular development (Allen and Cyster, 2008; Sukumar et al., 2008). FDCs characteristically possess long, delicate cytoplasmic extensions which form a reticular network in close contact with adjacent lymphocytes. A particular striking feature of FDCs is their ability to trap and retain antigen in the form of immune complexes on the surface of their dendrites for long periods of time, which serves as a repository of unprocessed antigen (Tew et al., 1982; Tew and Mandel, 1979). The ability of FDCs to trap and retain antigen and infectious virus within germinal centres for periods of more than a year (MacLennan, 1994) in a stable conformational state, and their intimate association with B cells, is crucial for an effective humoral immune response (Haberman and Shlomchik, 2003). A number of different pathologically relevant proteins, organisms and their products have been shown to be retained on FDCs in lymphoid tissue, for example; human, feline and simian immunodeficiency virus (Toyosaki et al., 1993; Ward et al., 1987; Tenner-Rácz et al., 1985), bovine viral diarrhoea virus (Fray et al., 2000), murine leukaemia virus (Siegler et al., 1973; Hanna et al., 1970; Szakal and Hanna, 1968), vesicular stomatitis virus (Bachmann et al., 1996), porcine circovirus (Hansen et al., 2010), tetanus (Kosco-Vilbois, 2003) and disease-associated prion proteins (McGovern and Jeffrey, 2007). Using laser capture microdissection (LCM), combined with quantitative real-time reverse transcription polymerase chain reaction (rRT-PCR), immunohistochemical analysis and in situ hybridization, we have shown in cattle that FMDV is maintained in the light zone of germinal centres for up to 38 days post-contact infection (Juleff et al., 2008). During these studies FMDV genome or proteins were not detectable in sites other than lymphoid tissue after resolution of clinical signs. Despite thorough investigation of twenty-two infected cattle, we were unable to isolate live virus from lymphoid tissue or determine sites of replicating virus after recovery from acute infection. We have reproduced these observations in sheep after the acute stages of infection. In addition, we have identified structural and non-structural viral proteins, indicating replicating virus, in palatine tonsil crypt epithelium of sheep at 21 days post-infection in the presence of high titres of neutralising antibody (unpublished data). Recently, we carried out a small field trial in the Kruger National Park and collected post-mortem samples from wild buffalo. We analysed the tissue samples by LCM and rRT-PCR and readily detected FMDV genome within germinal centres (unpublished data).

FDC-trapped human immunodeficiency virus has been shown to represent a significant reservoir of infectious and highly diverse virus, demonstrating greater genetic diversity than most other tissues, providing drug-resistant and immune-escape quasispecies that contribute to virus transmission, persistence and diversification (Keele et al., 2008). Furthermore, in contrast to other HIV reservoirs, where each infected cell harbours on average one virus, FDCs have the potential to trap and retain multiple, genetically diverse, replication-competent virus particles on their surface (Keele et al., 2008). Retention of intact FMDV particles on the FDC network would therefore provide an ideal mechanism of maintaining a highly cytopathic and lytic virus like FMDV extracellularly in a non-replicating, native, stable non-degraded state (Smith et al., 2001; Tew and Mandel, 1979). This reservoir could serve as the source of genetically diverse viral mutants (quasispecies), detected in carrier animals (Domingo et al., 2002; Vosloo et al., 1996). Susceptible cells that come into contact with the FDC network and the immune-complex coated bodies (Szakal et al.,