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Short communication

In vitro activity of VEGF-E produced by orf virus strains isolated from classical and severe persistent contagious ecthyma

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Abstract

Proliferative orf virus infections in adult sheep have increased in Italy in the past few years: these extreme cases are frequently fatal and difficult to differentiate from other infectious diseases of sheep such as blue tongue. A probable explanation for the proliferative and highly vascularized nature of the lesions was found in the expression of the VEGF-E gene encoded by the orf virus. To investigate a possible role of the viral VEGF in the pathogenesis of severe persistent orf virus lesions, the activity of four VEGF-E variants was compared by an angiogenesis in vitro model. Similar angiogenic activity was found between strains isolated from the classical and the proliferative forms of the disease, even if the latter was able to develop a higher number of vessels during the first 24 h of infection. Our in vitro findings seems to exclude that the VEGF variants encoded by the strain isolated from the atypical form of the disease could be the responsible for the histopathological aspect of the proliferative lesions.
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1. Introduction

The orf virus is the type species of the Parapoxvirus genus and causes a highly contagious pustular dermatitis in sheep, goats and humans (Haig and Mercer, 1998). The infection originates in damaged skin and the lesions progress through the stages of erythema, papulae,

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vescicles, pustulae and scab, with the infection confined to the skin and without any evidence of systemic spread (Mckeever et al., 1988). The lesions are remarkable for an extensive vascular proliferation and dilation as well as marked proliferation of the epidermis, with finger-like projections of the epidermis into the dermis (Groves et al., 1991). In the past few years, outbreaks of severe contagious ecthyma affecting adult sheep have increased in Italy. The lesions are extensive and proliferative and unlike classic orf do not spontaneously regress. Proliferative orf lesions have been reported in animals and humans (Mayet et al., 1997; Di Marco et al., 2000; Smith et al., 2002) and it is uncertain to what extent the virulence of certain strains could contribute to the severity of the disease; however, there is some evidence that at least the viral VEGF contributes to the proliferative and highly vascularised nature of the lesions. The orf virus VEGF, designated VEGF-E (Meyer et al., 1999) is encoded in the earlier phases of infection and shares some of the functional features of mammalian VEGF including the ability to stimulate the proliferation of endothelial cells and to promote vascular permeability (Wise et al., 1999; Savory et al., 2000; Shibuya, 2003). Most orf virus isolates carry NZ2-like VEGF gene with a high rate of sequence variation. Despite sequence variation, structural prediction of viral NZ2-like VEGFs were demonstrated to be similar to each other (Mercer et al., 2002). This study was carried out to further prove that a conservation of functional motifs correspond to a conservation of the activity and to investigate a possible role of the viral VEGF in the pathogenesis of severe persistent orf virus lesions.

2. Materials and methods

2.1. Cells and virus

Four orf virus strains were used in this study: the NZ2 strain (kindly provided from Dr. Andrew Mercer, Otago University, Dunedin, NZ), was isolated from naturally infected sheep in New Zealand; IT C2 and IT To were isolated in Italy from naturally infected lambs and chamois; the IT 01 strain was isolated in Italy from adult sheep with severe persistent proliferative lesions. All the viruses were adapted to grow in ovine testis cells (TFO) and derived from a different number of cell culture passages; in particular IT To and NZ2 are highly attenuated strains obtained after multiple cell culture passages.

2.2. Preparation of viral conditioned medium

Viral conditioned medium (CM) was prepared following the method described by Savory et al. (2000). Briefly, TFO cells were infected at a multiplicity of infection of 0.1 PFU per cell of each viral strain and incubated at 37 °C with 5% CO_2; a mock-infected cell cultures was also prepared as negative control. The supernatants were collected after 24 h of infection and after 100% cytopathic effect (CPE) was reached, three to four days post infection (PI). The cells were sedimented at low speed and the supernatants were filtered twice (0.1 μm pore-size filters) to remove infectious orf virus particles. To test the absence of virus, the CMs were tested by PCR (Inoshima et al., 2000). The mock-infected and infected monolayers (24 h PI) were prepared for RNA extraction using Tri reagent (Sigma–Aldrich, Inc., Germany) following the manufacturer's instructions. The RNAs were quantified by spectrophotometry and the ratio of 260/280 was estimated. The purified RNA was then incubated for 30 min at 37 °C with DNAse I (Amersham Biosciences Corp., USA) to eliminate genomic DNA.

2.3. Expression of VEGF-E mRNA in infected cell monolayers

A real time RT PCR assay was developed to allow absolute quantification of the viral VEGF mRNA. A previously amplified orf virus VEGF gene from the NZ2 strain, was cloned into the pCR 4/TOPO vector using TOPO cloning kit (Invitrogen, CA) and purified with Turbo Kit (QBIOgene, USA). The recombinant plasmid was linearized upstream the target sequence using the restriction endonuclease PmeI (Fermentas Inc., USA) and quantified by electrophoresis in 1% agarose gel containing ethidium bromide using 2-log DNA ladder (New England BioLabs Inc., UK). Ten-fold dilutions of the plasmid were made representing 2.25×10^9 to 2.5×10 copies of DNA/μl of template. The PCR standard curve (Wellmann et al., 2001) was constructed by plotting the plasmid DNA dilutions against the corresponding QPCR threshold cycle value. The RNA purified from each infected monolayer was

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