Summary

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Interaction of Orf virus with human keratinocytes and dermal fibroblasts: abortive infection and inhibition of Intercellular adhesion molecule-1 expression

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Introduction: Orf virus (ORFV) is an epitheliotropic virus with worldwide distribution, which infects replicating skin cells of small ruminants through skin lesions and causes pustular dermatitis. Even though the main hosts are small ruminants, ORFV can enter the broken skin of humans with close contact to infected animals and induces a usually localized infection ("milker's nodule"), which resolves without scarring after several weeks. Even though numerous case studies provide some insights, the knowledge about lesion development in humans and the role of the outnumbering cells in the skin, namely keratinocytes and dermal fibroblasts, remains limited.

Aims of study: Aim of this study was to characterize ORFV infection of human skin cells, regarding viral replication, cell death, and activation. Especially viral regulation of intercellular adhesion molecule (ICAM)-1, which mediates T cell adhesion, was analysed.

Material and methods: For the *in vitro* infection model, primary human keratinocytes and dermal fibroblasts were isolated from juvenile foreskin. After expansion, isolated primary skin cells were infected for 2 hours with ORFV followed by rinsing to remove non-adherent viral particles. Analyses were performed at 0, 6, 24, 48, 72 and 96 hours post infection. Cell morphology after infection was assessed using light microscopy and the degree of cytotoxicity was quantified using lactate dehydrogenase assay and flow cytometry. The viral load was determined using tissue culture infective dose 50 ($TCID_{50}$) following the formula of Spearman and Kärber and with flow cytometry. Expression of ICAM-1, was analysed using flow cytometry. Furthermore, the concentration of the pro-inflammatory cytokines, interleukin (IL)-6 and IL-8, in the cell culture supernatant was determined using enzyme-linked immunosorbent assay. The data from this study were acquired from 3-8 independent experiments with one donor each. Statistical evaluation was done by 1way ANOVA with Dunnett's multiple comparison test as post test, when values were equally distributed. Kruskal-Wallis-test with Dunn's Multiple Comparison test was applied to compare analyses of different groups of non-

parametric data. Within normally distributed samples, one sample t-test was done to compare normalized values. Within non-parametric normalized data, Wilcoxon signed ranked test was used. Two-tailed Student's t-test was used to evaluate data of two groups with normally distributed data. Correlational studies were done using Spearman correlation.

Results: Microscopical observation of infected keratinocytes and dermal fibroblasts revealed early rounding and detachment of keratinocytes (6 h), whereas in dermal fibroblasts these morphological changes occurred at a later time point (96 h). Rounding and detachment of cells were a consequence of cell death, which was shown by lactate dehydrogenase assay and flow cytometric analysis. Even though both investigated cell types could take up viral particles, the replication capacity and production of viral proteins remained low. Furthermore, secretion of IL-6 and IL-8 was limited to keratinocytes. A higher expression of ICAM-1 could be detected in infected keratinocytes and dermal fibroblasts cultures. This effect was transferable by virus-free supernatant from infected cells in keratinocytes, indicating involvement of soluble mediators. Interestingly, the expression of ICAM-1 on keratinocytes was limited to non-infected cells, on infected keratinocytes ICAM-1 was not detectable. This phenomenon occurred as well in dermal fibroblasts with low constitutive ICAM-1 expression. Even incubation with interferon-γ, a strong ICAM-1 inducer, could not upregulate ICAM-1 expression on infected keratinocytes, hinting to a strong blockade of ICAM-1 by ORFV.

Conclusion: These results reveal novel insights in human ORFV infection and characterizes the infection of human keratinocytes and dermal fibroblasts as abortive, in human keratinocytes accompanied with massive cell death. Furthermore, ORFV induced effects on ICAM-1 regulation were found pointing to a novel viral immune evasion mechanism.