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Characterization of the immune cells response and ultrastructural study of dendritic cell Golgi Apparatus role in ORF virus infection

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Contagious Ecthyma is an acute skin anthropozoonosis caused by orf virus (ORFV), which affects sheep and goat. The infectious agent is an epitheliotropic, double-stranded DNA poxvirus. Infection happens via the hurt skin, and causes a localized virus production in the epidermal cells and keratinocytes. This paper characterize the cellular immune response by cytochemistry in ORFV infection and studies the role of Golgi Apparatus (GA) of keratinocytes by transmission electron microscopy (TEM) and 3D models.

Twenty cutaneous biopsies in sheep from ORFV infected lesions were fixed in 10% formalin and embedded in paraffin for light microscopy. Paraffin sections were immunocytochemically stained (DAKO LSAB+/HRP) to identify major histocompatibility complex (MHC) II, CD1, CD79a, CD3, TCRγδ, and CD21. Furthermore, a portion from the biopsies was fixed with 1% glutaraldehyde/0.1M Na-cacodylate buffer, post-fixed in cacodylate buffer/1% osmium tetroxide and embedded in Durcupan for TEM. Ultrathin sections were observed and marked for MHC II with 10 nm gold particles. Three-dimensional computer-generated GA models were reconstructed from serial ultrathin sections.

Light microscopy images showed keratinocites positive for MHC II and negative for CD1, supporting the idea that Langerhans cells start the skin immune response. Positive cells for TCRγδ/CD3, CD21 and CD79a suggested the prevailing attraction for dendritic cells instead of T and B cells. Transmission electron micrographs revealed poxvirus-like particles. The images of GA showed the presence of large structures with bud profiles in the process of fusing with a Golgi stack and appeared emptying its ORFV content into it. ORFV induced swelling of Golgi vesicles, and perforation and fragmentation of GA, leading to a disorganized structure. Lateral vesicles close the Golgi stack were positive for MHC II, but in heavy infected cells the Golgi structures positive for MHC II were dispersed into the cytoplasm. An increased distance between GA and cell nucleus in infected cells was detected. Computer 3D reconstructions clarify the dynamics of GA in ORFV infected cell.

Overall, these results suggest that ORFV targets the vesicular export machinery and the structure and function of the GA might aid to escape cellular immune recognition.

Key words

Orf Virus, Golgi Apparatus, MHC II.