# POLARIZED MACROPHAGES USE VEILED SURFACE STRUCTURES TO INGEST HIV PARTICLES

**Podschwadt P[1], Mostafa H[1], Sander P[1], Schneider M[1], Zirafi O[2], Rode S[2], Münch J[2], Walther P[3]**

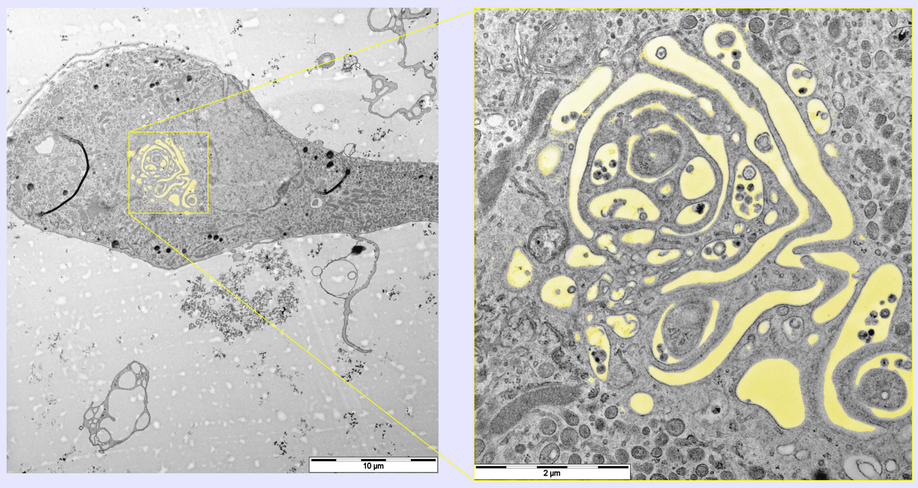
*Section Experimental Anaesthesiology, University Hospital Ulm*
*Institute of Molecular Virology, Ulm University Medical Center*
*Central Facility for Electron Microscopy, Ulm University*

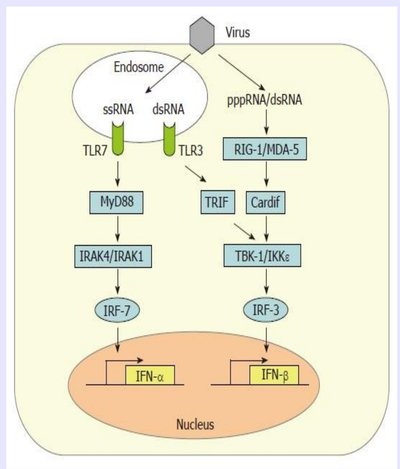
## Introduction

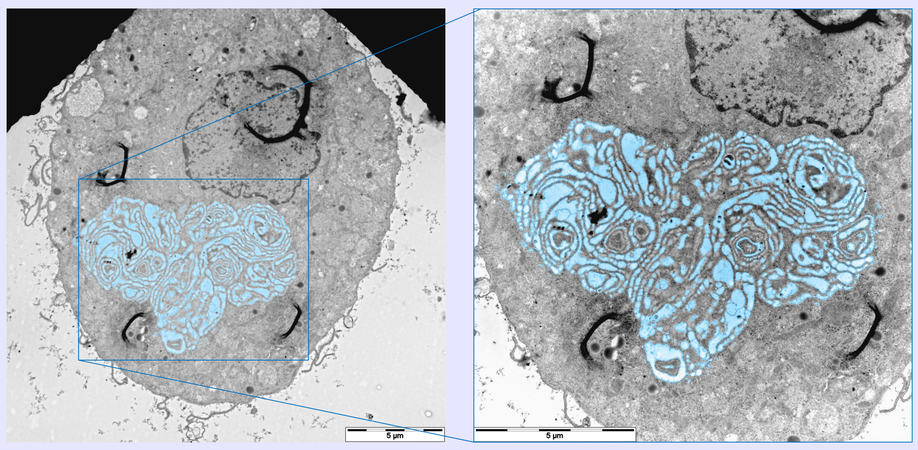
We established a culture system to characterize the inflammatory immune response of monocytes and dendritic cells in various diseases such as severe sepsis, diabetes type 2, autoinflammatory disorders such as macrophage activation syndromes (MAS), and from conditions with major tissue repair activities following trauma. After in vivo exposure to local growth factors, pro-inflammatory cytokines, and microbial compounds, these cells differentiate into macrophages and dendritic cells (Tacke and Randolph 2006). While these monocytes play a key role in eliminating invading bacteria, viruses, fungi, and protozoans, they can also play a role in the pathogenesis of inflammatory and degenerative diseases. In the present investigation we studied virus-uptake mechanisms by in vitro cultures macrophages and dendritic cells derived from patients. We used recombinant HIV-particles enriched from HEK-cell cultures and simultaneously tested the effect of a novel albumin-derived peptide, EPI-X4 (Mohr *et al.* 2015).

Current investigations imply the importance of endosomal/macropinocytotic uptake mechanisms in macrophages and dendritic cells by veiled surface structures. Macropinocytosis must be further studied for vaccination protocols to be applied for virus infections and malignancies.

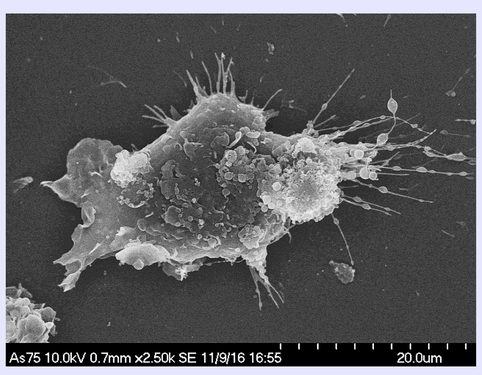
## HI Virus Uptake and Pathologic Antigen presentation in Inflammatory Macrophages (M1, M2c)

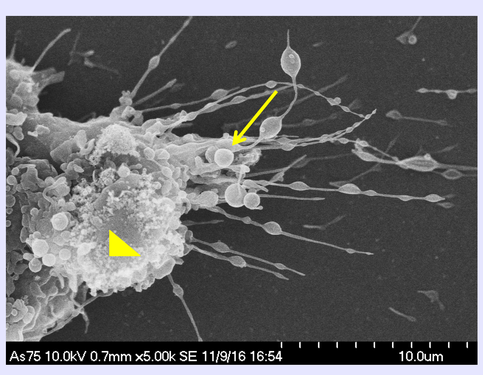



**Hyperinflammation Impairs Antigen Presentation.** Macrophages of the M2 type subtypes derived from the different diseases and disease states were all similar regarding a unique virus uptake structure characteristic composed of veiled plasma membrane leaves. These protrusions were preferentially used as virus uptake machinery and remained intact when ingested into the phagocytic cell. Remnants of the extracellular fluid in the cytoplasm were regularly observed which indicates a mechanism described as macropinocytosis. When HSA-treated cultures (Fig. B) were compared with non-treated cultures (Fig. A), the amount of morphologically identifiable particles was in all cases reduced. Most pronounced effects by HSA-treatment were observed in M2d type macrophage cultures

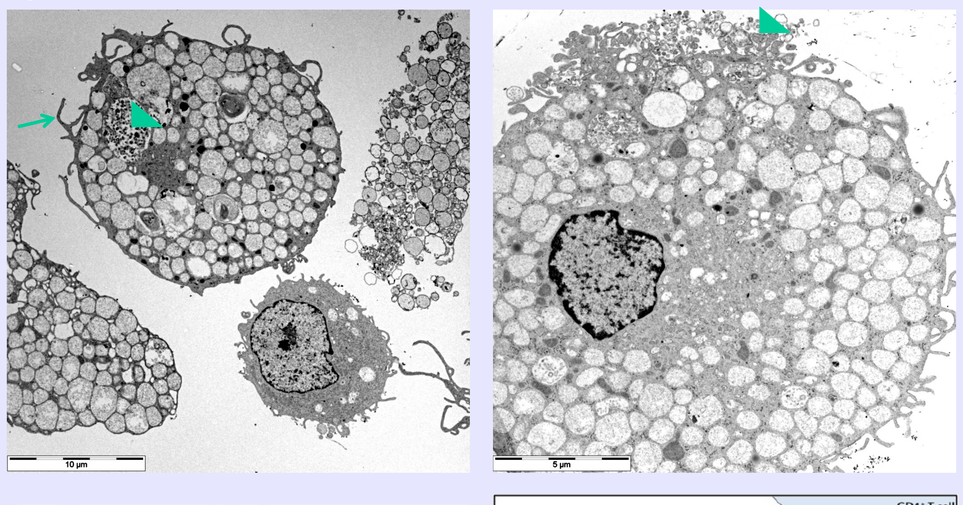


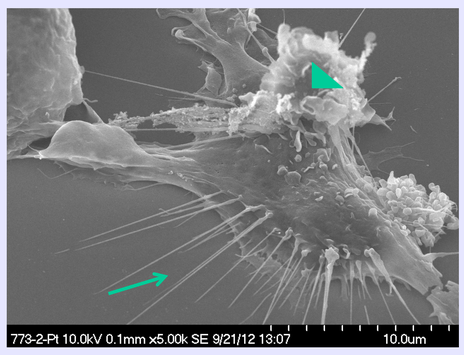




Sanning micrographs of **HI Virus** treated M2 macrophages, note the surface alterations of the retraction zone (arrow), and ruffled areas of the surface involved in virus uptake.(arrow head)

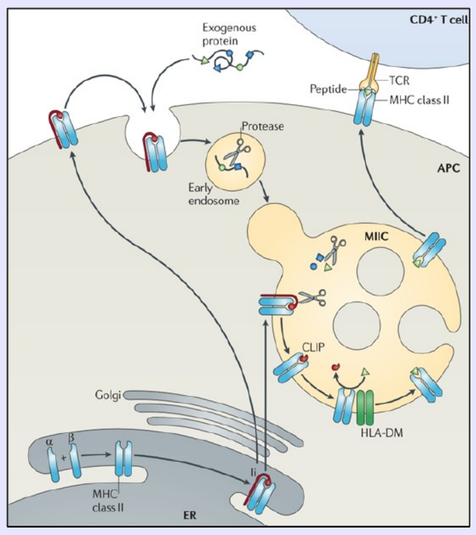
HSA (albumin) treatment attenutees **HI V**irus uptake in inflammatory macrophages

## Efficient Mechanism of Uptake and Antigen Presentation by Dendritic Cells

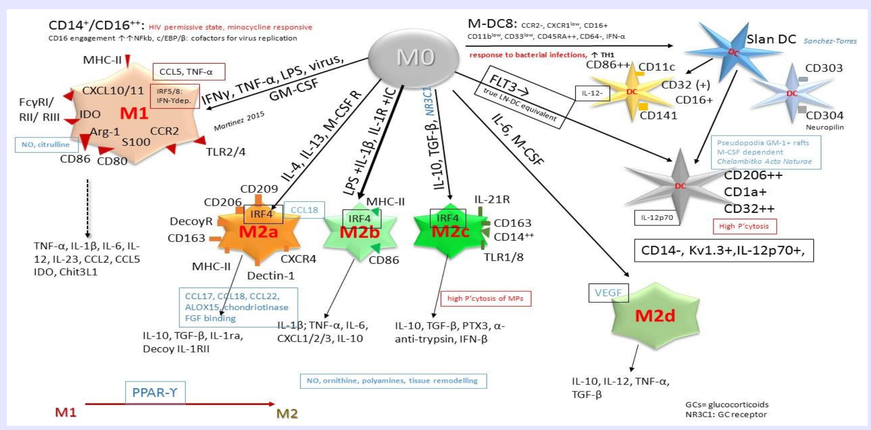



**Antigen processing and MHCII loading pathway.** MHCII molecules are synthesized after which they get loaded with antigens in a specialized compartment. Under influence of cathepsin activity and chaperone proteins (HLA-DM), the CLIP protein is exchanged by an antigenic peptide. The antigen loaded MHCII reaches the cell surface, activating CD4+ T cells. (Schematic by Crauwels 2014)




## Differentiation of Antigen Presentation Cells: Working Hypothesis




## Conclusion

**HI Virus treated antigen presenting cells derived from patients with inflammatory diseases are ineffecient antigen presenters:**

- **Virus uptake structures are different from classical endosomal or macropinocytotic vesicles**
- **Blebbing occurs in the retraction zone of M2 macrophages,**
- **Surface area of the antigen uptake presents with extensive blebbing as well and lack typical veil formation**
- **Humans serum albumin appears to downmodulate HI Virus uptake in inflamamtory macrophages possibly by interacting with CXCR4 (Zirafi et al. 2015)**

## References

Tacke, F., Randolph, G. J. 2006. Migratory fate and differentiation of blood monocyte subsets. *Immunobiology* 211:609-618.
Mohr, K. B., O. Zirafi, M. Hennies, S. Wiese, F. Kirchhoff, and J. Munch. 2015. Sandwich enzyme-linked immunosorbent assay for the quantification of human serum albumin fragment 408-423 in bodily fluids. *Analytical biochemistry* 476:29-35.
Peter Crauwels 2014. The interaction of *Leishmania major parasites* with human myeloid cells and its consequence for adaptive immunity.
http://www.wjgnet.com/esps%5CPub%5C10.3748%5Cv13%5Ci36%5CWJG-13-4818-g001.jpg