Endothelial derived extracellular vesicles express high level of HLA class II molecules under inflammatory conditions

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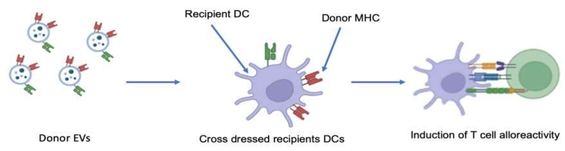
Introduction and aim

Extracellular vesicles (EVs)

EVs are nanoparticles produced by various cell types, playing a major role in cell communication.



In animal transplantation models, EVs have been shown to be a major mediator of allorecognition by transferring of intact donor MHC molecules, particularly class II, onto recipient dendritic cells, a process called cross dressing.

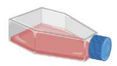
Endothelial cells (ECs)

The first cells encountered by recipient's immune system after solid organ transplantation is the endothelium of the donor organ.

Aim

This study evaluated the characteristics of EVs released from human ECs under normal or inflammatory conditions.

Methods



Stimulation of Human ECs with IFN-γ for 3 days

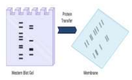
Collection of serum free media from culture of unstimulated and IFN-γ stimulated cells.

EVs isolation

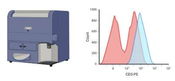
Size exclusion chromatography

EVs characterization

Size distribution; nanoparticle tracking analysis

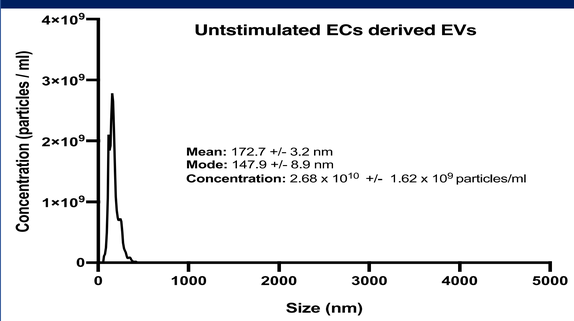


EV markers: Western blot



EV Protein surface markers: Flowcytometry

Results



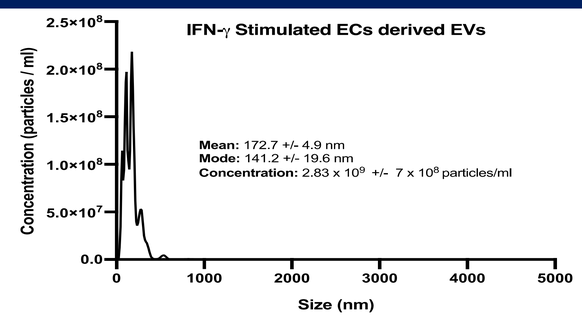


Figure 1. Less than 200 nm of size distribution was observed confirming the isolation of EVs from unstimulated and IFN-**γ** stimulated ECs.

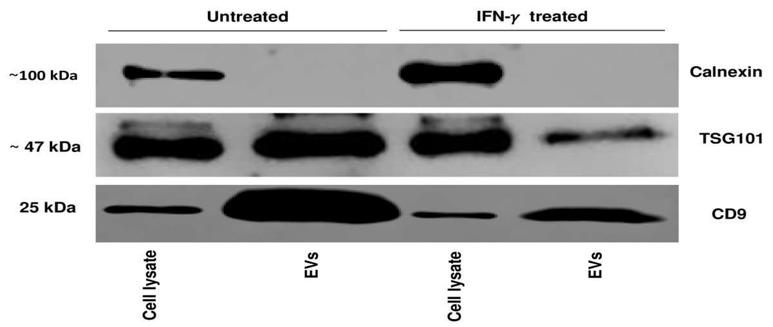


Figure 2. EV markers, including CD9 and TSG101 were shown on western blot with the absence of cellular contamination marker, calnexin.

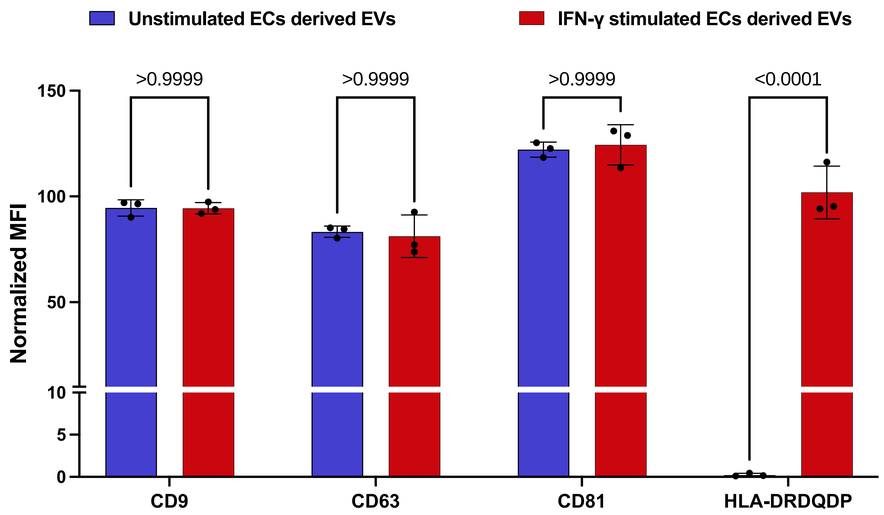


Figure 3. Isolated EVs from unstimulated and IFN-**γ** stimulated ECs showed a similar expression of EV markers (CD9, CD63 and CD81). HLA class II on the surface of IFN- **γ** stimulated ECs derived EVs showed a significant increase in the median fluorescence intensity (MFI) compared to those from unstimulated ECs.

Conclusion

- Our results indicated that EVs derived from human ECs under inflammatory conditions showed a high expression of HLA class II on their surfaces.
- The high expression of HLA class II on endothelial derived EVs could be a major part of allorecognition through cross dressing of recipient dendritic cells, leading to triggering of the adaptive alloimmune response.

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