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| Philipps-Universität | Institut für Lungenforschung | Hans Meerwein-Str. 2 | 35043 Marburg |  | DZL_logo_gekuerzt**iLung - Institut für** **Lungenforschung**  Univ.-Prof. Dr. Bernd T. Schmeck *Institutsdirektor*  Tel.: 06421 – 28 - 65713  Fax: 06421 – 28 - 21993  E-Mail: bernd.schmeck@uni-marburg.de  Anschrift: Hans-Meerwein-Str. 2  35043 Marburg  Web: www.i-lung.de  Az.: scb |
|  | | Marburg, |

Dear Teena Mehta,

We would like to thank you for the invitation to publish our work in JoVE. Therefore, we would like to suggest the manuscript “Analyzing the influence of *Legionella pneumophila* outer membrane vesicles” by Anna Lena Jung, Kerstin Hoffmann, Christina E. Herkt, Christine Schulz, Wilhelm Bertrams, and Bernd Schmeck for possible publication in JoVE.

We consider it to be suitable, because of the recently rising interest in outer membrane vesicles generated by Gram-negative bacteria. The manuscript describes a detailed protocol in the growth and handling of *Legionella pneumophila*, an important human pathogen causing mainly pneumonia, and the purification of the secreted outer membrane vesicles. Additionally, we describe here the following downstream analysis of the vesicles in stimulation and infection experiments of human and murine macrophages.

We could show – in conclusion – that OMVs from *L. pneumophila* are initially potent pro-inflammatory stimulators of macrophages, acting via TLR-signaling, while at later time points, OMVs facilitate *L. pneumophila* replication. OMVs might thereby pave the way for subsequent bacterial replication and hence promote spreading of *L. pneumophila* in the host.

We think that it will drive research forward because – besides the known pathways – we explored one other intriguing possibility to transfer virulence features to host cells, even over distance: Outer membrane vesicles that are released by *Legionella*. The described protocol can be adapted to the analysis of outer membrane vesicles from other Gram-negative bacteria. The standardization of protocols is required to obtain results that are comparable in different studies.

All authors have read and agreed to the manuscript. They declared to have no conflict of interest. The contribution of all authors, the original data, and the funding sources are provided.

We hope that you will find merit in our manuscript and are looking forward to your critical reviews.

Sincerely,

Anna Lena Jung Bernd Schmeck