Death Ligands Are Upregulated in Influenza Pneumonia and Contribute to the Apoptotic Depletion of the Resident Alveolar Macrophage Pool

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INTRODUCTION: Resident alveolar macrophage (rAM) depletion is a key event upon influenza virus (IV)-induced pneumonia. The mechanisms driving rAM loss are largely unknown, therefore, we aim to investigate the cellular crosstalk and the role of death ligands in IV-induced rAM depletion.
METHODS: C57BL/6 wild-type and TNFSF14-/- mice were intratracheally infected with influenza virus (PR/8) for flow cytometry analysis of the bronchoalveolar lavage. For apoptosis inhibition experiments, mice were daily injected with a caspase-3 inhibitor, a caspase-8 inhibitor, or a DMSO control. Transcriptional regulation of tumor necrosis factor superfamily (TNFSF) members and their receptors (TNFRSF) was assessed by gene expression analysis and by qPCR. Naïve rAM were treated with UV-inactivated BAL from infected mice and/or a caspase inhibitor for 24h and were subsequently subjected to colorimetric survival analysis.
RESULTS: Significant rAM depletion begins on day 3 post-IV infection, with rAM numbers reaching their lowest level on day 7, as shown by FACS analysis. Gene expression and FACS analysis further reveal that apoptosis is the main pathway driving rAM death. Apoptosis inhibition through caspase blocking in vivo attenuates rAM depletion and leads to a reduction in weight loss. This protective effect is mirrored by abrogated apoptosis induction upon day-7-BAL and caspase-3 or caspase-8 inhibitor treatment of naïve rAM in vitro. Pro-apoptotic TNFSF members, including TNFSF10 and TNFSF14, are upregulated in day 7 flow-sorted rAM. Of note, TNFRSF14 is the highest upregulated TNFSF receptor in these samples, while its ligand, TNFSF14, is upregulated in the lung homogenate of infected mice as early as day 2 post-infection. Interestingly, treatment of naïve rAM with recombinant TNFSF14 stoichiometrically increases cell death. In accordance with that, significantly increased rAM survival is observed in TNFSF14-/- mice on day 7 after IV infection.
CONCLUSION: Apoptosis-related rAM depletion upon IV infection can be attenuated by caspase inhibition leading to an improved clinical score. In the context of death-ligand-initiated rAM apoptosis, TNFSF14 appears as a promising target, as it can directly reduce rAM survival in vitro, is highly upregulated in the inflamed mouse lung on a ligand-and-receptor level and its depletion leads to protection of the rAM pool in vivo.

Keywords : Influenza, macrophages, apoptosis, death ligands
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