CHARACTERISING THE IMMUNOTHERAPEUTIC CAPABILITIES OF BACTERIAL OUTER-MEMBRANE VESICLES

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Background Outer-membrane vesicles (OMVs) are highly immunogenic particles shed by Gram-negative bacteria, utilised for a variety of functions from nutrient acquisition to antibiotic resistance. Their potent immunogenicity, in combination with an inability to replicate, has led to these vesicles being developed as a novel class of vaccines. Increasing evidence also suggests that the innate immune activation stimulated by OMVs can facilitate the recognition and destruction of malignant cells, inducing a sustained elimination of tumours in various animal models. At present however, the mechanistic pathways underlying the anti-tumour response remains poorly understood. Herein, we sought to investigate OMV-mediated immune interactions, elucidating key cells able to be leveraged in the context of a potential immunotherapy.

Methods OMVs were isolated from a hypervesiculating E.coli K-12 MG1655 strain expressing penta-acylated LPS, achieved via pal and lpxM deletions. Co-cultures were performed using peripheral blood mononuclear cells (PBMCs) from healthy donors, with cytokine and cell marker expression determined using ELISA and flow cytometry. Negatively-isolated OMV-activated lymphocytes were co-cultured with various cancer cell lines, and cytotoxicity investigated using the MTS assay and flow cytometry.

Results Our findings demonstrate that MG1655 Δpal ΔlpxM OMVs induce a broad increase in activation markers on NK cells, αβ T cells and γδ T cells. We observed a concordant release of IFN-γ and granzyme B, suggesting the cells exhibit a cytotoxic phenotype upon OMV stimulation. γδ T cells were found to be the predominant cell type to proliferate, expanding from 3% to 40% of the total lymphocyte population. Noticeably, the majority of γδ T cells were of the Vγ9Vδ2 type, which possess the ability to respond to both bacterial metabolites as well as stress markers present on malignant cells. Since Vγ9Vδ2 T cells present an MHC-independent innate-like activation mechanism, they are well positioned to respond to OMV stimulation whilst maintaining oncolytic capabilities. Indeed, we observe robust cytolytic activity of Vγ9Vδ2 T cells against both breast cancer and leukaemia cell lines (SkBr3 and Nalm6 respectively) after OMV-mediated expansion. These data therefore identify Vγ9Vδ2 T cells as able to directly respond to OMV-stimulus whilst maintaining anti-tumour capabilities.

Conclusions Our findings support the hypothesis that Vγ9Vδ2 T cells are a crucial component of the OMV-mediated anti-tumour immune response, cells that may be used to improve future immunotherapies.

REFERENCE

Ethics Approval Human PBMC were purchased from Cambridge Bioscience. According to their site, Cambridge Bioscience source human material in partnership with Research Donors, an HTA-licensed clinic, based in London, dedicated to the collection and processing of human blood and fresh leukopaks for research purposes. Research Donors is ISO 9001 2015 certified with Research Ethics (REC) approval as a Research Tissue bank, and participates in the UK NEQAS QA scheme.