Delivery of TLR7 agonist to monocytes and dendritic cells by DCIR targeted liposomes induces robust production of anti-cancer cytokines.

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Publication date: 2016

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Delivery of TLR7 agonist to monocytes and dendritic cells by DCIR targeted liposomes induces robust production of anti-cancer cytokines


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Tumor immune escape is today recognized as an important cancer hallmark and is therefore a major focus area in cancer therapy. Monocytes and dendritic cells (DCs) are central to creating a robust anti-tumor immune response and establishing an anti-tumorogenic microenvironment, but are directly targeted by the tumor escape mechanisms to develop immunosuppressive phenotypes. Providing activated monocytes and DCs to the tumor tissue is therefore an attractive way to break the tumor-derived immune suppression and reestablish cancer immune surveillance.

Our aim was to activate monocytes and DCs with high efficiency. To that end we investigated an immunotherapeutic Toll-Like Receptor (TLR) agonist delivery system comprising liposomes targeted to the dendritic cell immunoreceptor (DCIR) and examined the targeting properties of the liposomes as well as their immune activating potential in blood-derived monocytes, myeloid DCs (mDCs), and plasmacytoid DCs (pDCs).

Peripheral Blood Mononuclear Cells (PBMCs) were purified from buffy coats by gradient centrifugation and incubated with anti-DCIR agonist-immunoliposomes, non-targeted agonist-liposomes, agonist alone, or buffer and the association of the liposomes with PBMC subsets was analyzed by flow cytometry. Following a washing step, PBMCs were cultured for 5 days and on each day the secretion of cytokines was analyzed using Meso Scale Discovery (MSD) sandwich ELISAs.

Liposomes were 150 - 180 nm of size and anionic. The conjugation procedure was optimized to provide 50-60 mAbs per liposome. Flow cytometry showed that the anti-DCIR immunoliposomes showed strong preferential association to monocytes, mDCs, and pDCs (PBMCs). Non-targeted liposomes showed little association to any cell subset. Non-targeted liposomes showed little association to any cell subset (fig. 2). Non-targeted liposomes showed little association to any cell subset (fig. 3), while a the IgG-control immunoliposomes showed some uptake by unspecified mechanisms.

MSD ELISAs showed that the targeted delivery greatly increased the potency of the TLR7 agonist compared to the free drug. Further, incubation with the anti-DCIR immunoliposomes resulted in potent secretion of key anti-cancer cytokines (IL-12p70, IFN-γ and IFN-α) as well as the pro-inflammatory cytokines TNF-α and IL-6. Importantly, due to gradually decreasing secretion of IL-10, the important IFN-γ/IL-10 ratio increased over the 5 day assay period.

Conclusions

Monocytes and mDCs were targeted with high specificity over lymphocytes, and we observed potent TLR7-specific secretion of the anti-cancer cytokines IL-12p70, IFN-α 2a, and IFN-γ. This delivery system could be a way to improve cancer treatment either in the form of a vaccine with co-formulated antigen or as an immunotherapeutic vector to boost monocyte and DC activity in combination with other treatment protocols such as chemotherapy or radiotherapy.

References