Delivery of Tolerance Inducing Agents to Dendritic Cells

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Introduction. Dendritic cells (DCs) are phagocytes known to play a key role in the immune system[1]. Their expression of costimulatory and co-inhibitory molecules drives the antigen-dependent activation of T cells[2]. Therefore, directing maturation of DCs would allow for shaping the subsequent defensive immune responses in applications such as tissue engineering and autoimmune diseases. In this study we focus on the intracellular as well as extracellular delivery of tolerance inducing agents to DCs using poly(lactic-co-glycolicacid) (PLGA) microparticles. We will be exploring acetylsalicylic acid (ASA) as the intracellular component and indoleamine 2,3 dioxygenase (IDO) as the extracellular agent. As part of its anti-inflammatory effects, ASA hinders antigen presentation by DCs[2]. We hypothesize therefore that a depot of ASA will maintain immaturity of DCs, upregulate inhibitory signals, and prevent antigen presentation to T cells. IDO on the other hand, catalyzes the rate limiting step of tryptophan catabolism into N-formyl-kynurenine. Depletion of this essential amino acid is believed to increase susceptibility of T cells to apoptosis while some of its resulting metabolites (quinolinic acid and 3-hydroxyanthranilic acid) have a direct cytotoxic effect on effector T cells resulting in reduced immune activation[3]. In addition, IDO-expressing cells preferentially induce proliferation of regulatory T cells.

Methods

PLGA microparticles were fabricated using a single emulsion/evaporation technique with encapsulation of ASA in the oil phase followed by characterization including size distribution, loading efficiency and release kinetics. The microparticles were incubated with murine myeloid derived dendritic cells at a 10:1 ratio and necrosis and apoptosis assessed using flow cytometry. The levels of co-stimulatory molecules (CD80, CD86, CD40), MHC-II and co-inhibitory molecules (PD-L1/PD-L2) were also measured using flow cytometry. IDO was purified from E.coli and its activity as well as endotoxin levels were measured using a ChromoLAL assay. The protein was then added to the cell culture media at different concentrations and maturation markers (CD80, CD86, MHCII) were assessed via flowcytometry.

Results

Herein, we present our ability to obtain an ASA loading efficiency of approximately 40%, a release profile showing a burst of ASA in the first six days followed by sustained release over a period of forty days and decreased levels of CD80, CD86 and MHCII markers on dendritic cells (**Figure 1**)

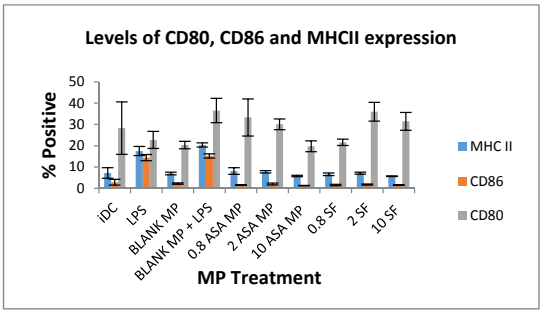

Figure 1. Levels of MHC II, CD86 , CD80

Our preliminary data also shows IDO does not increase the levels of co-stimulatory molecules or MHCII on DCs (**Fig. 2**)

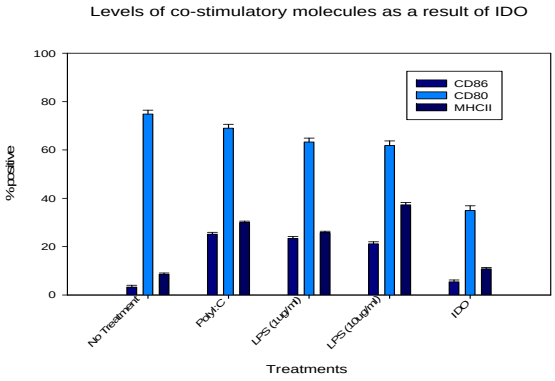

Figure 2. Levels of maturation markers of DCs exposed to IDO.

Conclusion

From this study we can conclude that PLGA microparticles are able to load significant levels of ASA and release it at a constant rate over a period of forty days. We have shown that the delivery of ASA using a particulate system decreases the levels of costimulatory molecules as well as MHC-II. We can also concluded IDO does not act as a damage-associated molecular pattern or pathogen-associated molecular pattern molecule. Future directions include studying the effects of our systems on T cells.

References

[1]Jhunjhunwala,S. J. Control Release 2009;133(3):191-7 [2] Muzammal H. Int. Immunopharmacology 2012;12:10-20.) [3]Mellor, A.L. et al Immunol.**168**, 3371-3776 (2002)

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