Adjuvant Optimization Using Cellular Arrays for Vaccine Development

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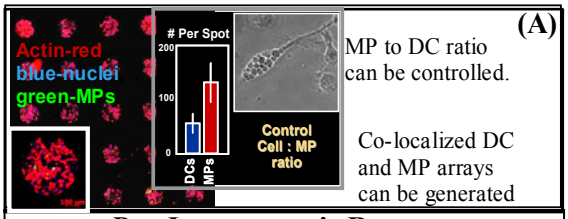
Introduction: High-throughput (HTP) screening of synthetic vaccines incorporated in a targeted drug-delivery vehicle for cells modulating the immune response is attractive for drug development and discovery. Although there are now scores of known antigenic epitopes and adjuvants present, there has not emerged an analogous systematic examination of the functional responses of immune cells toward combinatorial vaccine formulations in a HTP-manner. To address this concern we have developed cellular microarray co-localizing vaccine microparticles with dendritic cells, a primary target for such vaccines. Further we have utilized this method to screen adjuvants for both immunogenic and suppressive dendritic cell responses for either targeting cancer and infectious diseases or autoimmune disorders.

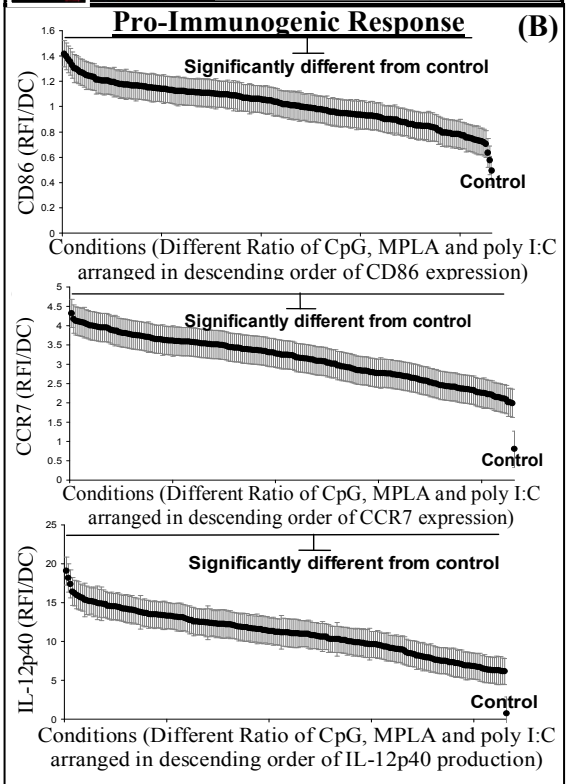
Methods: Bone marrow-derived dendritic cells (DCs) were arrayed and cultured on poly lactide co-glycolide microparticle (PLGA-MP) encapsulated with different factors (see below), printed on chips in a square matrix. The DCs were then immunofluorescently stained for activation or tolerogenic markers and quantified using image analysis.

Results: A) DCs are co-localized and cultured on the printed particle arrays and MP to DC ratio can be controlled. DCs were cultured on randomized arrays of poly I:C encapsulated MPs, stained and quantified for surface expression of MHC-II. It was observed that there was minimum cross-talk between the islands and the DCs expression of MHC-II follows the poly I:C gradient (data not shown). These experiments were performed to validate the chip-construct in order to screen vaccine components. Furthermore, experiments were performed to optimize the MP to DC ratio on per island basis that the DCs can phagocytose in 24 h. This ratio was determined to be 10:1 (data not shown). **B)** DC activation quantified via MHC-II, CD86 cell surface expression, IL-12p40, IL-10 cytokine production and chemokine receptor expression were modulated and best combination of poly I:C, MPLA and CpG adjuvants identified. This combination will be utilized in vivo. **C)** DC IDO-expression, a measure of tolerogenic extent can be quantified on the chip and best combination of tolerance identified. The next step is to perform in vivo experiments to corroborate in vitro data.

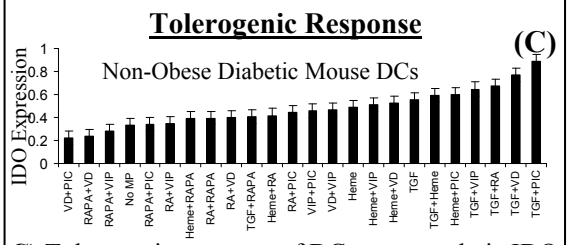
Conclusion: Co-localized microparticle/dendritic cell arrays enable screening of immune cell responses to vaccine particles. Furthermore, both immunogenic and tolerogenic drug combination in different dilutions can be screened in a high-throughput fashion.

Reference: Acharya AP et al 2008, Acharya AP et al 2009. Acharya AP et al 2010.





B) Particles encapsulated with combinations and dilutions of CpG, MPLA and poly I:C induce differential DC activation in an additive format. Furthermore, the DC expressions of activation markers are quantified in situ.



C) Tolerogenic response of DCs measured via IDO expression has been modulated using combination of different tolerance inducing drugs with TGF + poly I:C combination resulting in the highest IDO expression.

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