Effects of different TLR Agonists on in vivo cDC1 and in vivo cDC2 Activation

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Background

- Mature dendritic cells (DCs) contribute to the postinflammatory regulatory immune system response through antigen uptake, antigen presentation, cross-presentation, and T cell priming.
- Classical DC differentiation is largely promoted in vivo by the toll-like receptor 3 (TLR3).
- Toll-like receptors (TLRs) including TLR3 and TLR7/8 activate multiple arms of the immune response and promote the activation of type 1 and type 2 classical dendritic cells (cDC1s and cDC2s, respectively).
- cDC1s are XCR1+ and are known to respond to TLR3 stimulation; cDC2s are CD172α+ and are known to respond to TLR7/8 stimulation.
- It is unknown to what extent cDC1s and cDC2s can be activated by unconventional TLR stimulation, and whether combination treatment will alter their optimal activation state.
- Immune responses are mediated by costimulatory molecules CD40, CD80, CD86, which regulate antigen-specific T cell responses, and MHC I and MHC II, which mediate antigen presentation, and can be upregulated in response to TLR stimulation.

Hypothesis

We hypothesis that the Poly IC will more efficiently activate cDC1s, and Imiquimod will upregulate the activation of cDC2s. Additionally, combination treatment will lead to comparable levels of activation as individual agonist treatment.

Methods

Flow Cytometry. DC costimulatory expression levels of CD40, CD80, CD86, MHC I, and MHC II were examined post-stimulation using an extracellular stain. The method of staining is similar to FACS sorting, but cells were fixed with 4% formaldehyde prior to analysis. Activation was quantified using flow cytometry using the X20 LSR Fortessa.

Results (continued)

Our studies demonstrated that costimulatory expression increased over time for both cell types, and in vivo cDCs and cDC2s were most activated at 24 hours post-stimulation. cDC1s expressed higher basal levels of costimulatory molecules compared to cDC2s. cDC2s were most activated after stimulation with Poly IC, indicated by significantly higher expression of CD40, CD80, CD86, MHC I and MHC II after 24 hours, while Imiquimod failed to significantly increase cDC1 activation. Interestingly, combination treatment, for some maturation markers, showed lower activation compared to Poly IC alone. Poly IC did not increase cDC2 activation; however, Imiquimod or combination treatment significantly increased cDC2 activation, with upregulation of CD40, CD80, CD86 and MHC I at 24 hours post-stimulation.

Conclusions

This data demonstrates that cDCs and cDC2s are largely activated 24 hours after TLR agonist stimulation. Poly IC is most effective for promoting cDC1 activation, while the combination of Poly IC and Imiquimod is most effective for activating cDC2s. This, in turn, is effective to use Imiquimod to promote cDC1 activation, although there is a potential for Poly IC to further promote robust cDC2 activation, to increase cDC2 maturation in conjunction with Imiquimod. Further research would investigate the communication between T cells, cDC1s, and cDC2s after dendritic cell stimulation with Poly IC and Imiquimod to elucidate the effects of different agonists on the quality of the immune response.

References


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