A Novel Combination Adjuvant Specifically Targets Dendritic Cell Hyperactivation to Enhance T cell Responses

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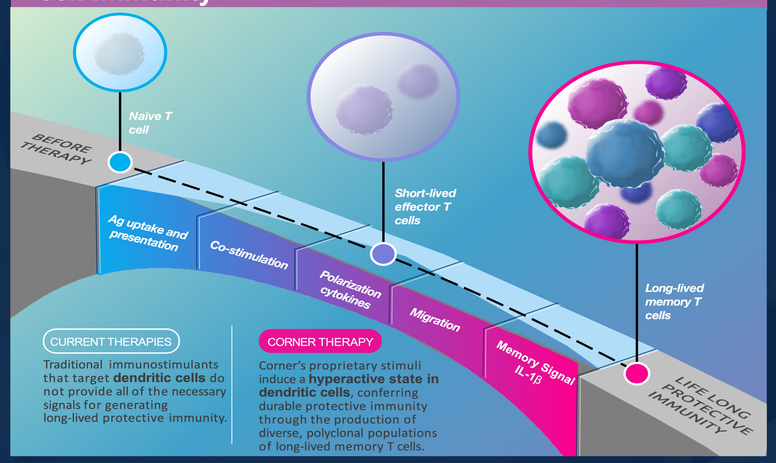
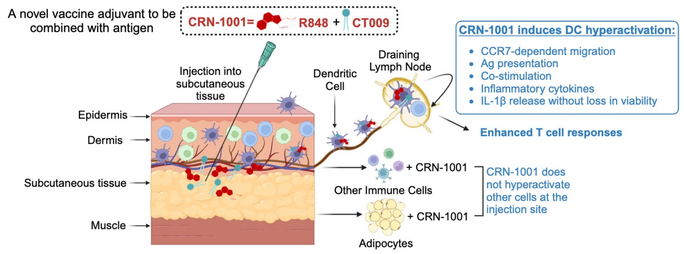
Five key functions of dendritic cells enable durable T cell immunity



Targeting dendritic cell hyperactivation using CRN-1001 as a vaccine strategy



Key Questions Being Addressed:

- What are the direct effects of CRN-1001 on dendritic cells?
- In the context of our proposed route of administration of CRN-1001 in a vaccine, does CRN-1001 act on other cell types at the subcutaneous injection site?
- What downstream effects result from dendritic cell hyperactivation?

CRN-1001 induces DC hyperactivation, leading to expression of cytokines and activation markers

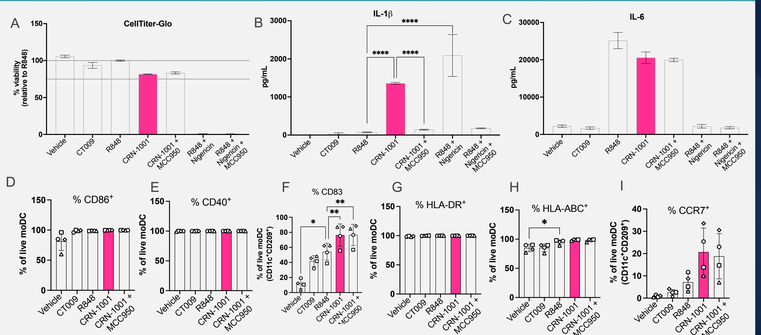


Figure 1: Human monocyte derived dendritic cells (moDCs) were treated for 24 hours with R848 and CT009 individually or in combination. To induce pyroptosis, cells were primed with R848 for 3 hours prior to stimulation with nigericin. (A) One day after indicated moDC treatments, ATP was measured using CellTiter-Glo as a measure of viability. Cell culture supernatant was used to measure (B) IL-1β and (C) IL-6. Flow staining was performed to assess expression of co-stimulatory molecules (D) CD86 (E) CD40, DC activation marker (F) CD83, MHC molecules (G) HLA-DR, (H) HLA-ABC and lymph node-homing chemokine receptor (I) CCR7.

After CRN-1001 treatment, moDCs secrete IL-1β in the absence of pyroptotic cell death, which is a needed signal for memory T cell formation. Additionally, hyperactivated moDCs maintain expression of inflammatory cytokines such as IL-6 and expression of cell surface proteins needed for T cell activation.

moDC hyperactivation using CRN-1001 induces hypermigratory behavior

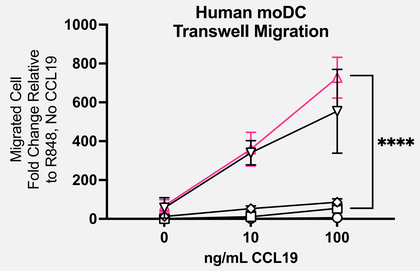


Figure 2: moDCs were tested *in vitro* for their migratory capacity using a transwell migration assay. moDC were treated with R848 and/or CT009 overnight, and then cells were plated in the apical chambers of transwells. In the basal chambers, media containing varying concentrations of CCL19, a ligand for CCR7, were added. After an overnight incubation, cells in the basal chambers were enumerated. These data represent one of three donors tested in a study. Similar results were obtained with the other two.

CRN-1001 treatment, enhances migratory ability of moDCs.

CRN-1001 does not impair human adipocyte viability nor induces adipocyte hyperactivation

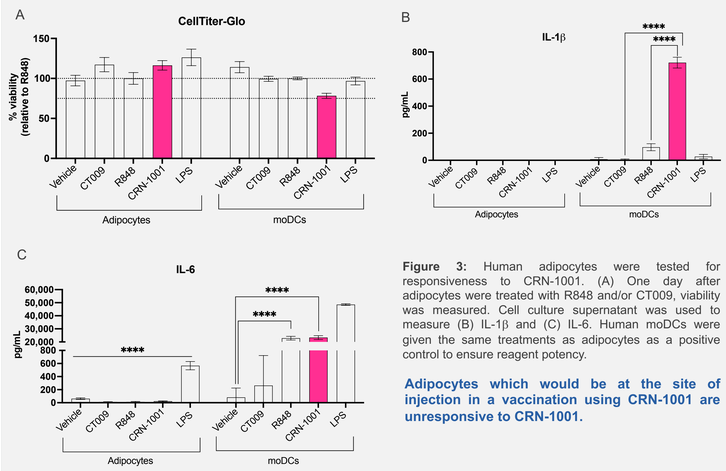


Figure 3: Human adipocytes were tested for responsiveness to CRN-1001. (A) One day after adipocytes were treated with R848 and/or CT009, viability was measured. Cell culture supernatant was used to measure (B) IL-1β and (C) IL-6. Human moDCs were given the same treatments as adipocytes as a positive control to ensure reagent potency.

Adipocytes which would be at the site of injection in a vaccination using CRN-1001 are unresponsive to CRN-1001.

Direct effects of CRN-1001 on other immune cell types are not observed

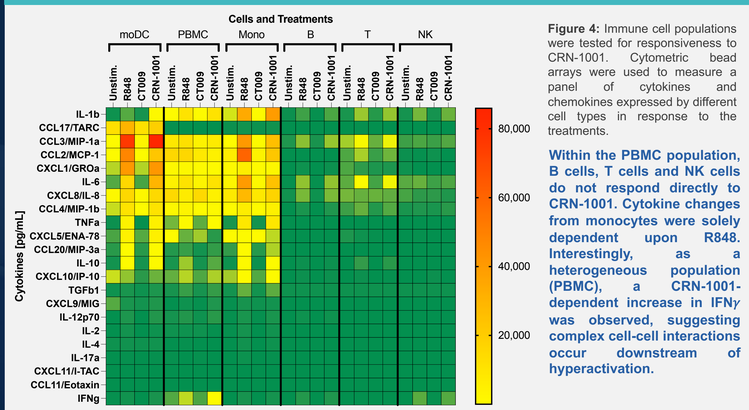


Figure 4: Immune cell populations were tested for responsiveness to CRN-1001. Cytometric bead arrays were used to measure a panel of cytokines and chemokines expressed by different cell types in response to the treatments.

Within the PBMC population, B cells, T cells and NK cells do not respond directly to CRN-1001. Cytokine changes from monocytes were solely dependent upon R848. Interestingly, as a heterogeneous population (PBMC), a CRN-1001-dependent increase in IFNγ was observed, suggesting complex cell-cell interactions occur downstream of hyperactivation.

DC hyperactivation using CRN-1001 enhances Th1 responses from CD4+ memory T cells

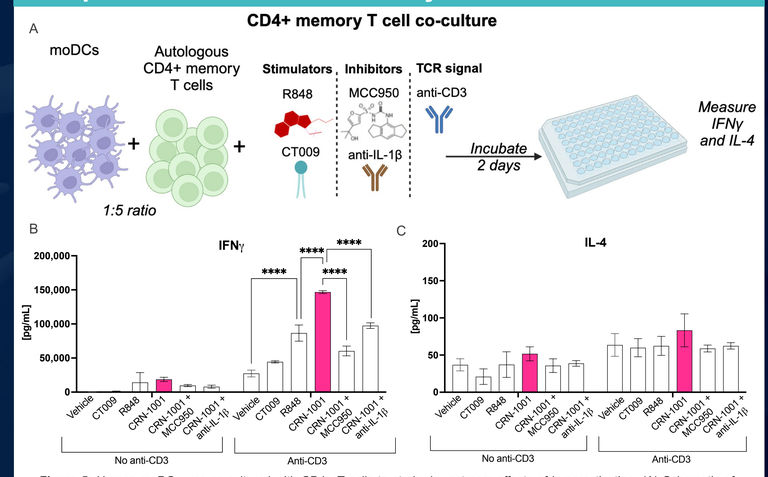


Figure 5: Human moDC were co-cultured with CD4+ T cells to study downstream effects of hyperactivation. (A) Schematic of the co-culture setup to assess memory CD4+ T cell responses in the presence of hyperactivated moDCs. moDCs were co-cultured with autologous memory CD4+ T cells stimulated with 0.1ng/mL anti-CD3. (B) IFNγ and (C) IL-6 were measured 2 days after coculture setup.

Memory CD4+ T cells cultured with hyperactivated moDCs increase secretion of IFNγ, suggesting an enhanced Th1 response. In contrast IL-4 levels remain unchanged, indicating that CRN-1001 does not drive Th2 responses. The increased IFNγ production by hyperactivated moDCs is IL-1β dependent, as indicated by conditions containing the inflammasome inhibitor MCC950 or anti-IL-1β blocking antibody.

DC hyperactivation using CRN-1001 leads to enhanced antigen-specific CD8+ T cell responses

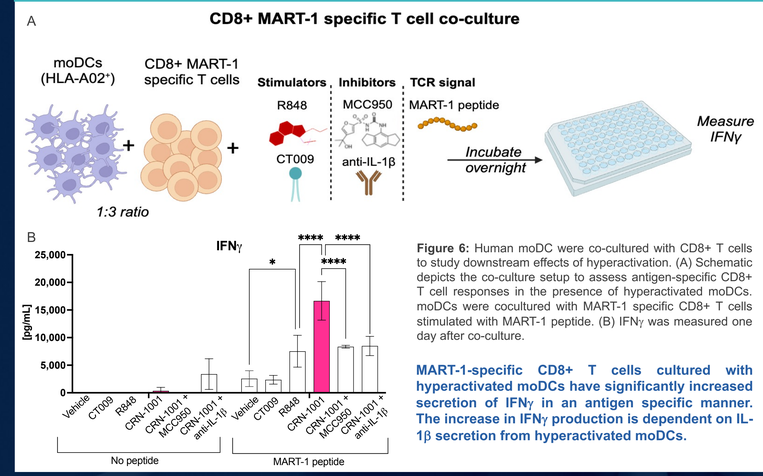


Figure 6: Human moDC were co-cultured with CD8+ T cells to study downstream effects of hyperactivation. (A) Schematic depicts the co-culture setup to assess antigen-specific CD8+ T cell responses in the presence of hyperactivated moDCs. moDCs were cocultured with MART-1 specific CD8+ T cells stimulated with MART-1 peptide. (B) IFNγ was measured one day after co-culture.

MART-1-specific CD8+ T cells cultured with hyperactivated moDCs have significantly increased secretion of IFNγ in an antigen specific manner. The increase in IFNγ production is dependent on IL-1β secretion from hyperactivated moDCs.

CONCLUSIONS

- In our planned use of CRN-1001 as a vaccine adjuvant, dendritic cells are hyperactivated, enabling increased antigen presentation, co-stimulation, inflammatory cytokine secretion, DC migration, and IL-1β production that is necessary to activate durable T cell responses.
- In the context of subcutaneous injection, other cells such as adipocytes, NK cells, and T cells at the injection site do not directly respond to CRN-1001 nor hyperactivate. Monocytes demonstrate cytokine changes that are driven by R848.
- Co-cultures of hyperactivated DCs with T cells demonstrate that CRN-1001 enhances IFNγ responses after activation of memory CD4+ T cells and antigen-specific CD8+ T cells via IL-1β signaling.