A Chemically Stable Adjuvant Hyperactivates Dendritic Cells to Initiate Durable Adaptive Immunity

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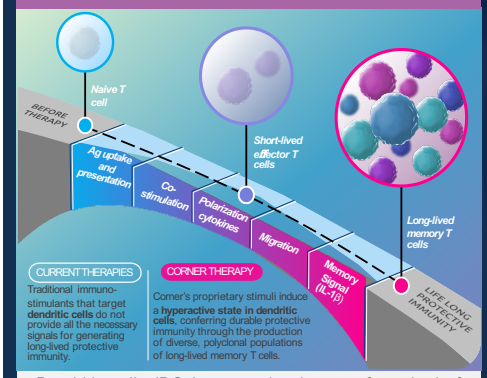
Five Dendritic Cell Activities Required for Memory T cell-mediated Immunity



- Dendritic cells (DCs) survey the tissues of our body for threats to the host such as cancer or infection.
- DCs are the only cells that can stimulate new T cell responses to cancer and infection by providing multiple signals: antigen in the context of MHC molecules, costimulatory proteins, and inflammatory cytokines.
- IL-1β is a critical cytokine released upon inflammasome activation that promotes potent, durable T cell responses.
- Hyperactivation is a cell state where living DCs hypermigrate to lymph nodes and secrete IL-1β in the absence of pyroptosis, enabling other critical functions as antigen presenting cells.
- Corner Therapeutics has developed a combination of adjuvants, R848 and CT009 (CRN-1001), for clinical use to hyperactivate DCs and promote durable T cell-mediated immunity.

Dendritic Cell Hyperactivation Induces a Unique Cell State

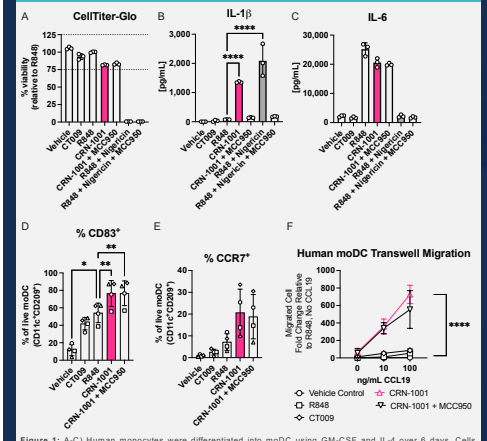


Figure 1: A-C) Human monocytes were differentiated into moDC using GM-CSF and IL-4 over 6 days. Cells were treated with the indicated stimuli all at once with the exception that nigericin was added after a 3 hour prime with R848. One day after treatment, cells were used to measure viability and supernatant was used to measure cytokine expression. Data from one representative donor of three in an experiment are shown. D and E) moDC were treated with the indicated stimuli for one day. Cells were then collected and stained for flow cytometry analysis. CD83 and CCR7 markers are shown as indicators of DC maturation state after treatments. Four healthy human donors were used for the study. F) Human moDC were treated overnight with indicated stimuli before being placed in the apical chamber of a transwell with 5μm pores. CCL19 which drives CCR7-mediated chemotaxis was added to the basal chamber, and cell migration to the basal chamber was quantified the next day. Representative data from one of three human donors in an experiment are shown. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

When treated with CRN-1001, human monocyte derived DCs express inflammatory cytokines and maturation markers. Additionally, CRN-1001 enhances migratory behavior and enables IL-1β secretion in the absence of pyroptotic cell death.

Hyperactivating Chemical CT009 is Highly Stable

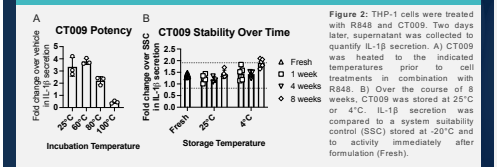


Figure 2: THP-1 cells were treated with R848 and CT009. Two days later, supernatant was collected to quantify IL-1β secretion. A) CT009 was heated to the indicated temperatures prior to cell treatments in combination with R848. B) Over the course of 8 weeks, CT009 was stored at 25°C or 4°C. IL-1β secretion was compared to a system suitability control (SSC) stored at -20°C and to activity immediately after formulation (Fresh).

CT009 is a highly stable molecule that can retain activity across a clinically relevant range in temperatures and storage times.

CRN-1001 Enhances Migration of Myeloid Cells

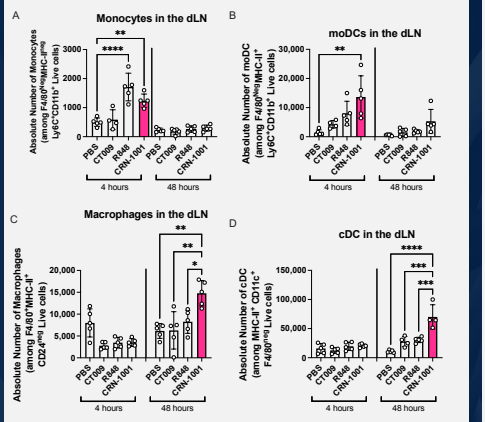


Figure 3: Combinations of R848 and CT009 were subcutaneously injected into B6 mice. Endogenous infiltrating A) monocytes, B) moDC, C) macrophages, and D) cDC in the draining lymph nodes (dLN) were quantified at 4 and 48 hours. Data points represent mice in study. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Injection of CRN-1001 induces the migration of endogenous myeloid cell populations to the draining lymph nodes. After 4 hours, R848 induces monocytes to drain to lymph nodes whereas moDCs require both R848 and CT009 signals. At 48 hours, CRN-1001 treatment enhances macrophage and cDC migration.

Hyperactive DCs Increase T Cell IFNγ Responses In Vitro

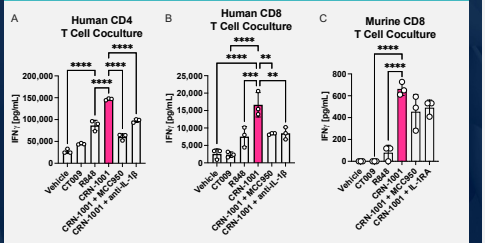


Figure 4: A) Human moDC were treated with the indicated stimuli and cocultured with autologous memory CD4 T cells. To induce a T cell signal 0.1ng/mL anti-CD3 was added to the culture. Two days later cell culture supernatants were collected to quantify IFNγ secretion by CD4 T cells. B) Human moDC from an HLA-A02* donor were treated with the indicated stimuli and cocultured with MART-1 Ag-specific CD8 T cells. MART-1 peptide was added to the cultures at 10ng/mL. One day later, cell culture supernatant was collected to quantify IFNγ secretion by T cells. C) Murine FLT3L-BMDC were treated with combinations of R848, CT009, MCC950, and OVA antigen overnight. Cells were then collected and cocultured with CD8 T cells isolated from Ova-immunized mice. If receiving IL-1RA, it was added at the time of coculture. Four days later, IFNγ was quantified from culture supernatants. Data points represent coculture replicates in studies. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

In vitro cocultures of hyperactivated DCs with T cells demonstrate that hyperactivation enhances the downstream IFNγ response from T cells. The increased IFNγ secretion is an effect of IL-1β produced by hyperactivated DCs and can be inhibited by blocking IL-1β secretion and signaling.

CRN-1001 Induces B and T Cell Responses In Vivo

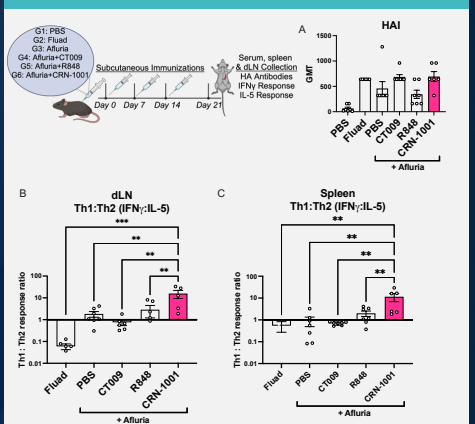


Figure 5: B6 mice were subcutaneously injected at weekly intervals three times to immunize against influenza. Fluad is a vaccine adjuvanted with MF59 whereas Afluria only contains inactivated flu virus. Afluria was adjuvanted with combinations of R848 and CT009. One week after the final immunization, sera, spleens, and dLNs were collected. A) Sera were used to measure hemagglutinin (HA) specific antibodies via a hemagglutination inhibition assay (HAI). Cells from B) dLNs and C) spleens were restimulated with Afluria for 3 days. IFNγ and IL-5 responses were measured via ELISA. Data points each represent one mouse in the study. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

By incorporating a clinically approved flu vaccine (Afluria) with CRN-1001, antibody responses can still be elicited. Additionally, CRN-1001 directs T cells to a Th1 response that is observable in both dLN and spleen.

CRN-1001 Induces a Unique Protective Response Against Lethal Flu Challenge

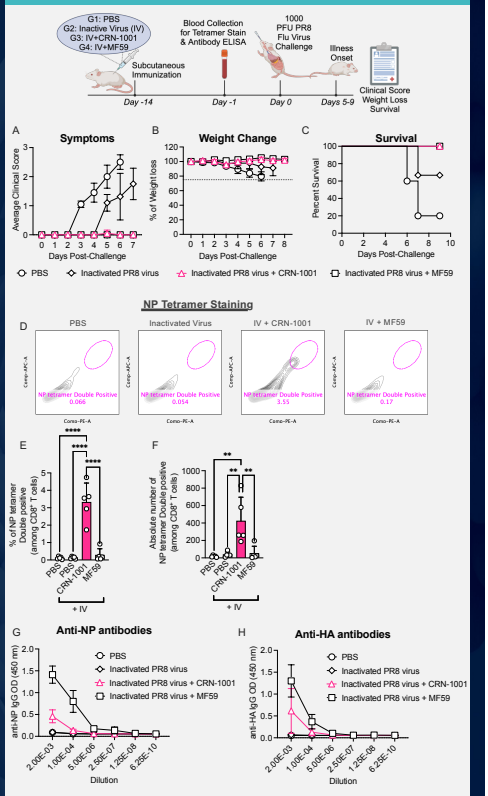
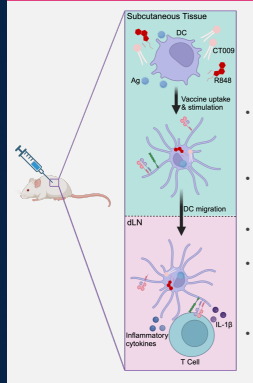


Figure 6: Balb/c mice were immunized with PR8 influenza inactivated virus (IV). As an adjuvant to this antigen, IV was combined with CRN-1001 or combined with the clinically used adjuvant MF59. Mice were immunized once prior to lethal virus challenge. One day prior to live virus challenge, blood was collected from half of each cohort to measure immune responses. The remaining half of each cohort were challenged with a lethal dose of PR8 virus. Mice were monitored for disease symptoms and survival. A) After virus challenge, mice were monitored and scored based on fur coat, lethargy, labored breathing, and openness of eyes on scales from 0-3 with healthy phenotypes scored as 0. Scores for a mouse were then averaged for a day. Averaged scores of a group are shown. B) Mice were weighed daily after infection and compared to their baseline weights before infection. C) Survival frequencies of mice after infection are shown. D) Blood collected after vaccination was stained for nucleoprotein (NP) specific CD8 T cells. Sample flow plots demonstrate the gating strategy where staining utilized the same tetramers but with two different fluorophores to increase specificity of the stain. E) Percentage of tetramer double-positive CD8 T cells was calculated. F) The absolute numbers of NP-specific CD8 T cells were quantified. G and H) Sera from mice were serially diluted and used in an NP and HA ELISAs to measure IgG. **p<0.01; ****p<0.0001

Using the mouse-adapted PR8 strain of influenza virus, mice were immunized using inactivated virus with or without CRN-1001 or MF59. Like other adjuvants, CRN-1001 induces an antibody response. Unique to CRN-1001 treatment, vaccinated mice expanded NP-specific CD8 T cells. Vaccinations using CRN-1001 protected mice from disease and lethality, and the immune response signature was unique from vaccination strategies currently used in the clinic.

Key Takeaways



- CRN-1001 stimulation results in a hyperactive cell state of DCs
- Hyperactivated DCs are highly efficient at migrating to dLN
- CRN-1001 induces IL-1β from living DCs
- Hyperactivated DCs maintain ability to present Ag, co-stimulatory signals, and other inflammatory signals
- Hyperactivated DCs activate a Th1 response

About Corner Therapeutics

Corner Therapeutics is a preclinical stage company pioneering a new class of multi-purpose immunotherapies that protect the young and old. Our versatile dendritic cell hyperactivation platform induces exceptional memory T cell responses that provide robust and durable immunity against a virtually unbounded range of cancers and infectious diseases. We are actively seeking talented individuals who are passionate about bringing transformational immunotherapies to the clinic to join our team. Please reach out if you are interested in learning about Corner and joining our mission.