A Unique Dendritic Cell Activation State Utilizes Inflammasomes to Stimulate Memory T Cell Responses in Humans

Jonathan Chow, Veronica Komoroski, Kelsey Finn, Emily Gosselin, Caitlin Sullivan, Carolyn MacFarlane, Sivan Elloul, Andrew Cornforth, Jonathan C. Kagan

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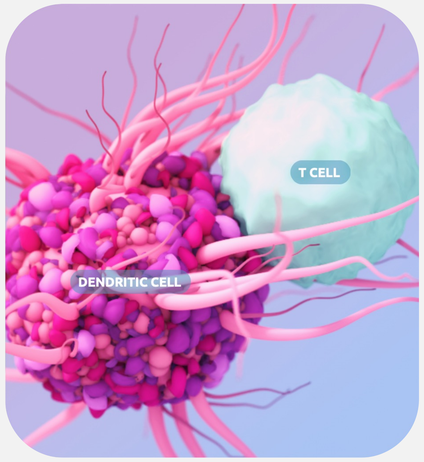
Dendritic Cells Determine T Cell Mediated Immunity



Dendritic Cells (DCs) are the apex regulators of the immune system.

- 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.
- Hyperactivation is a cell state when living DCs hypermigrate to lymph nodes and secrete IL-1β without pyroptosing, producing potent and durable T cell responses.

Introducing CTX42, a Potent Hyperactivator of Human DCs

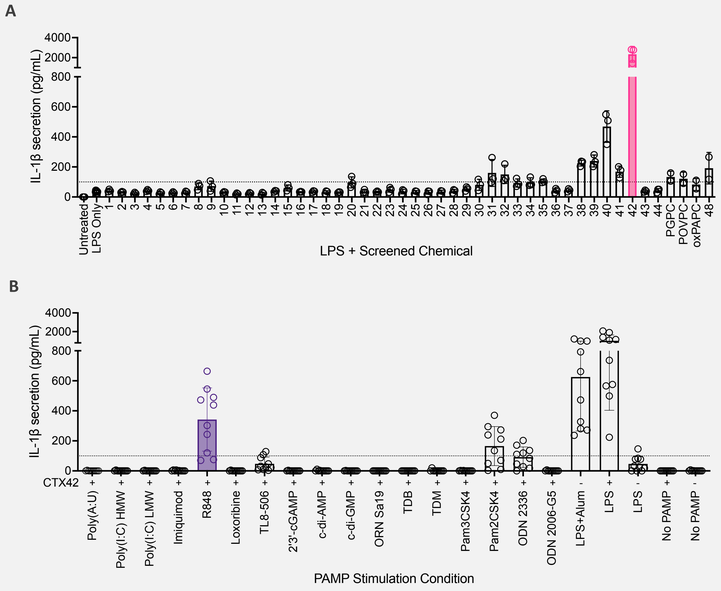


Figure 1. Chemical screening identifies clinically relevant pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that hyperactivate human monocyte-derived dendritic cells (moDCs). **A)** A library of chemicals were tested for their ability to induce IL-1β secretion while maintaining cell viability. moDC prepared from 3 donors were treated with LPS and candidate chemicals. Responses were compared to known murine DC hyperactivators: PGPC and oxPAPC. Supernatants were collected after 24h to measure IL-1β. **B)** Lead candidate CTX42 was combined with a panel of PAMPs to identify a clinically suitable substitution for LPS for hyperactivation of DCs. IL-1β was quantified one day after moDC stimulation. Data points represent the means of biological triplicates from 10 human donor samples.

Inflammasomes within Hyperactive DCs Contribute to Superior Immunostimulatory Activities

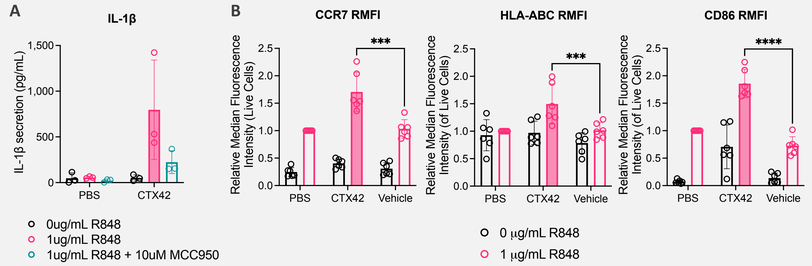


Figure 2. DC hyperactivation using clinically relevant molecules enhances DC activity. **A)** Human moDC from 3 donors were left unstimulated, activated with R848, or hyperactivated with R848 + CTX42. IL-1β release in live moDCs was NLRP3 inflammasome-dependent, as MCC950 inhibitor reduced IL-1β levels. **B)** After 24h of stimulation, human moDC from 6 donors were stained for activation markers. Hyperactivation increased expression of CCR7 (required for DC homing to lymph nodes), HLA-ABC (required for antigen presentation to CD8 T cells), and CD86 (costimulatory molecule for T cell activation).

Hyperactive DCs induce Th1 Cytokine Production from Naïve Human T cells

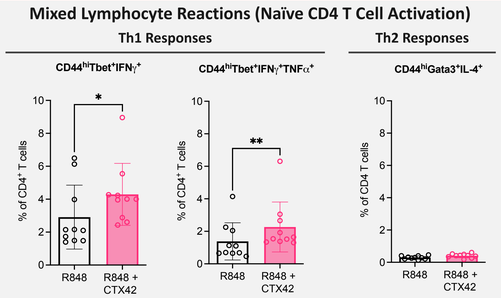


Figure 3. Mixed lymphocyte reactions reveal an improved polarization of naïve CD4 T cells towards a Th1 response when moDCs are hyperactivated. Human moDCs were either activated with R848 or hyperactivated with R848 + CTX42. After a 24h stimulation, naïve CD4 T cells from an allogeneic donor were added to the culture with IL-2. Five days later, cells were restimulated and intracellularly stained to investigate T cell polarization. Hyperactivation increases Th1 responses whereas Th2 responses were minimal. Ten human donor samples were tested.

IL-1β Production by Inflammasomes in Hyperactive DCs Maximizes Memory Th1 Cell Reactivation

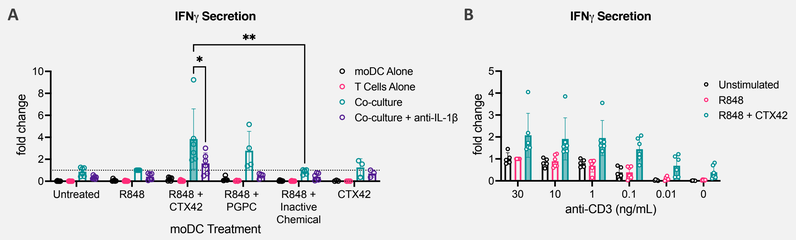


Figure 4. moDC hyperactivation enhances memory CD4 T cell reactivation. Human moDC from 6 donors were cultured with autologous memory CD4 T cells ($CD3^+CD4^+CD45RO^+$). **A)** When cultured with 0.1ng/mL anti-CD3 antibody as a TCR signal, reactivated T cells produce IFNγ. Hyperactivated moDC using R848 + CTX42 enhances the T cell IFNγ (Th1) response. Blocking IL-1β inhibits IFNγ production. **B)** Cocultures incubated with a range of anti-CD3 antibody concentrations simulate weak TCR signals. Hyperactivation enhances Th1 recall responses, overcoming suboptimal TCR signaling.

PBMC Stimulations Reveal Superiority of Hyperactivators over FDA-approved Immunostimulants

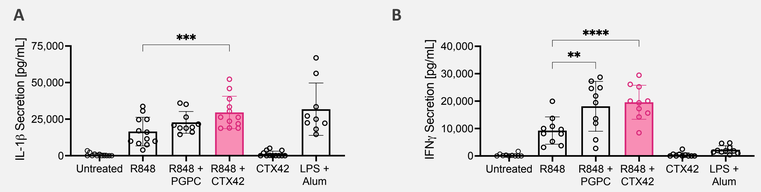


Figure 5. Hyperactivation stimuli applied to PBMC rapidly and easily assesses cellular crosstalk. PBMC were isolated from 10 fresh whole blood samples and stimulated for 2 days as indicated. **A)** Monocytes are the main responders to inputted stimuli, resulting in IL-1β release. Both hyperactivation (R848 + PGPC/CTX42) and pyroptosis (LPS + Alum) result in enhanced IL-1β secretion. **B)** Downstream, NK cells are hypothesized to respond to hyperactivated cells without antigen recognition, resulting in IFNγ production. Hyperactivation enhances IFNγ release. Note that pyroptosis stimuli (LPS + Alum) result in IL-1β release but cannot recapitulate IFNγ production.

CTX42 Derivatives have Enhanced Potency

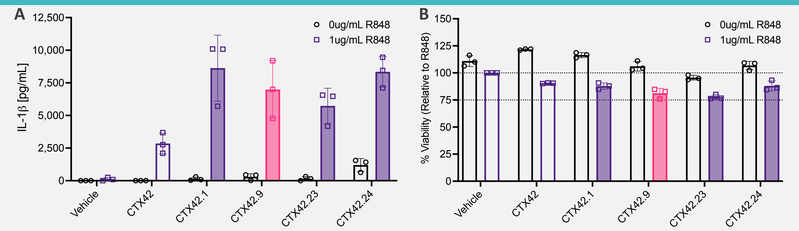
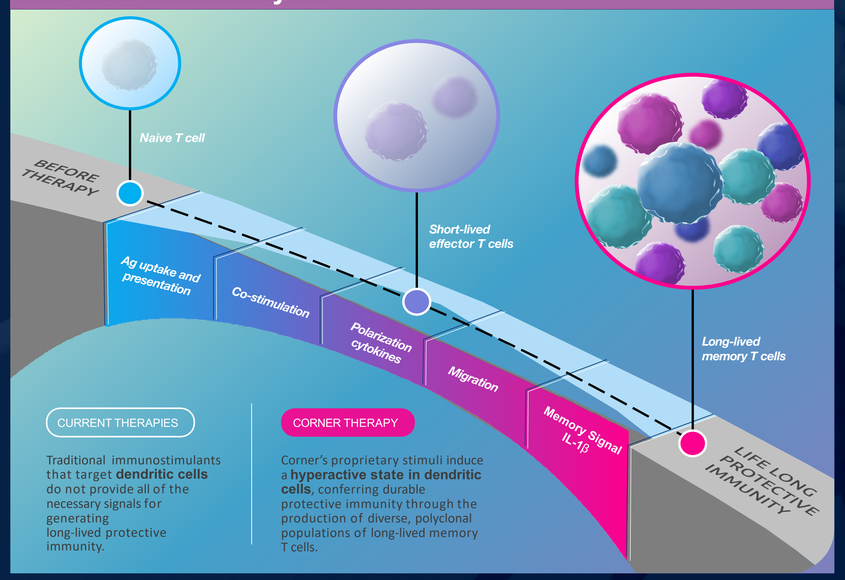


Figure 6. Derivatives of CTX42 increase hyperactivation potency. Derivatives of CTX42 were synthesized and tested for their ability to hyperactivate moDC from 3 donors. **A)** Enhanced IL-1β release was detected from multiple derivatives tested at equimolar concentrations at 24hr. **B)** Cell culture viability was assessed using CellTiter-Glo. Treatments had minimal effect on cell viability. CTX42.9 was selected for further study in mouse models.

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