Adjuvant That Hyperactivates Dendritic Cells Stimulates Robust T and B Cell Immunity to Influenza Antigens

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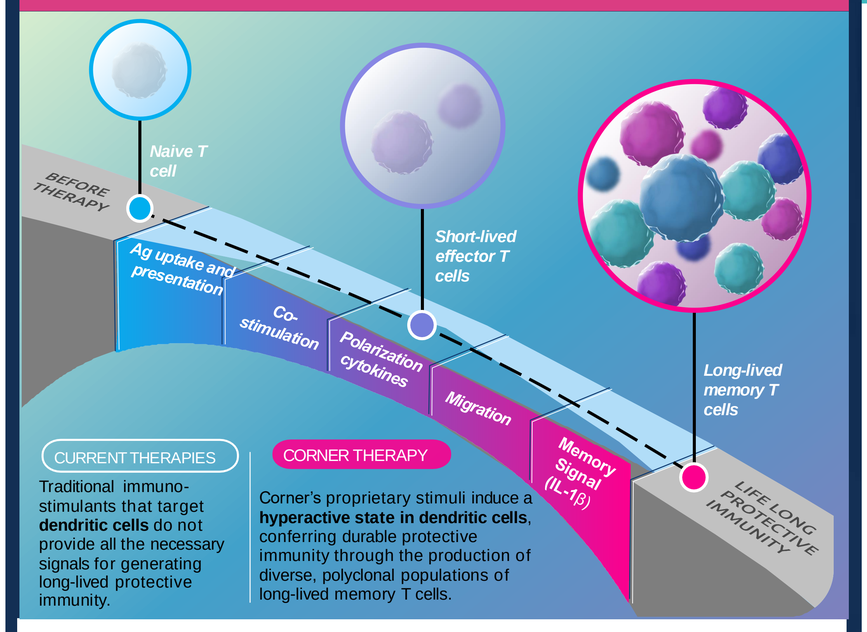
Five Dendritic Cell Activities Are Required for Memory T cell-mediated Immunity



- Adjuvants stimulate critical dendritic cell (DC) activities such as antigen uptake and presentation as well as expression of co-stimulatory molecules and T cell polarizing cytokines
- A new class of adjuvants recently defined as hyperactivators is able to induce these canonical DC activities but also enhances DC migration and allows for the secretion of I IL-1β from viable DCs, thus enhancing memory T cell formation
- Herein we evaluate a proprietary hyperactivating adjuvant, a combination of R848 and CT009 (CRN-1001), for its ability to stimulate DCs and drive antigen specific T and B cell responses in mice and non-human primates when combined with a commercially available influenza vaccine.

CRN-1001 Leads to DC Hyperactivation and IL-1β Secretion From Viable Cells

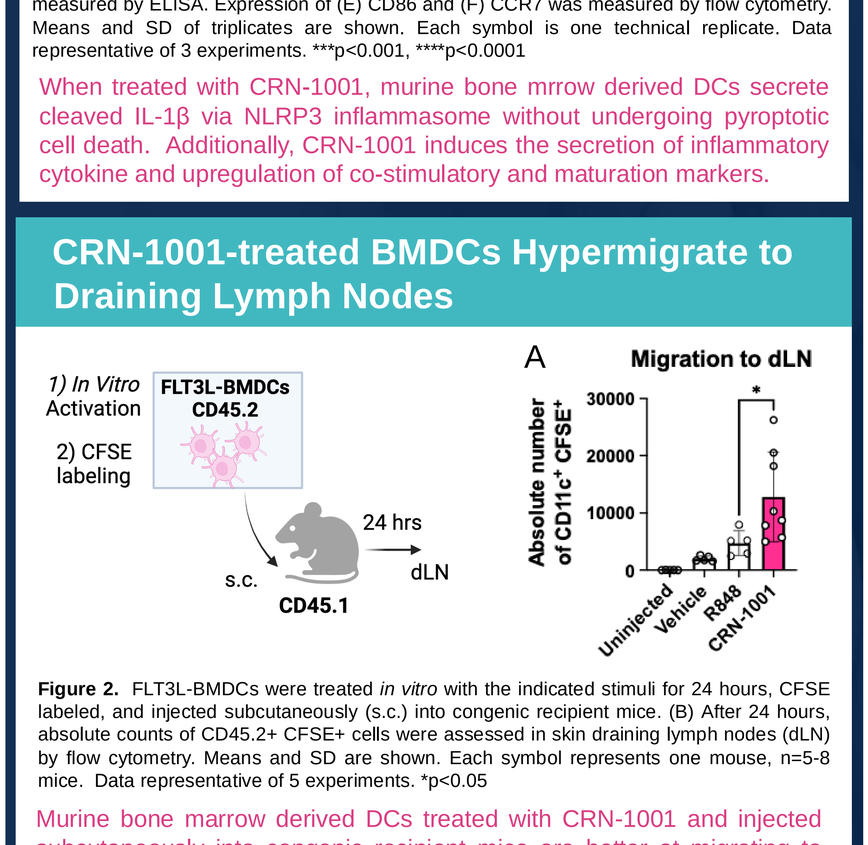


Figure 1: FLT3L-BMDCs were treated with the indicated stimuli for 48 hours. (A, C) Cleaved IL-1β was measured by Lumit, (B) viability was assessed via Cell-Titer Glo, (D) TNFα was measured by ELISA. Expression of (E) CD86 and (F) CCR7 was measured by flow cytometry. Means and SD of triplicates are shown. Each symbol is one technical replicate. Data representative of 3 experiments. ***p<0.001, ****p<0.0001

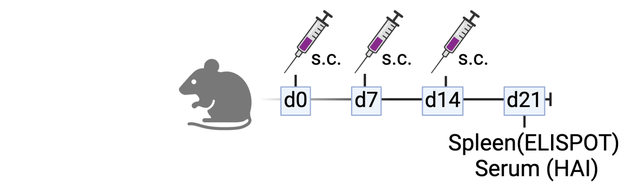
When treated with CRN-1001, murine bone mrrow derived DCs secrete cleaved IL-1β via NLRP3 inflammasome without undergoing pyroptotic cell death. Additionally, CRN-1001 induces the secretion of inflammatory cytokine and upregulation of co-stimulatory and maturation markers.

CRN-1001-treated BMDCs Hypermigrate to Draining Lymph Nodes

Figure 2. FLT3L-BMDCs were treated *in vitro* with the indicated stimuli for 24 hours, CFSE labeled, and injected subcutaneously (s.c.) into congenic recipient mice. (B) After 24 hours, absolute counts of CD45.2+ CFSE+ cells were assessed in skin draining lymph nodes (dLN) by flow cytometry. Means and SD are shown. Each symbol represents one mouse, n=5-8 mice. Data representative of 5 experiments. *p<0.05

Murine bone marrow derived DCs treated with CRN-1001 and injected subcutaneously into congenic recipient mice are better at migrating to skin draining lymph nodes, suggesting increased opportunities for DC-T cell interactions.

CRN-1001 Induces a Superior Th1 Response Compared to Commercial Influenza Vaccines



Afluria Quadrivalent: Non-Adjuvanted Commercial Influenza Vaccine

Fluad Quadrivalent: MF59-Adjuvanted Commercial Influenza Vaccine

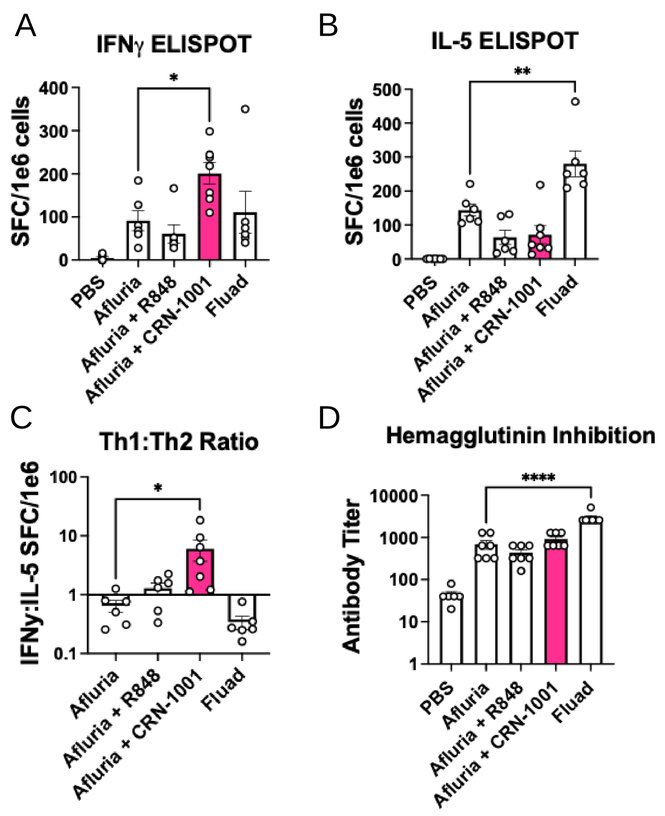


Figure 3: B6 mice were immunized subcutaneously weekly with Afluria and CRN-1001. For comparison, mice were also injected subcutaneously with Fluad. On day 21, antigen-specific T cell responses were assessed in the spleen by (A) IFNγ ELISPOT and (B) IL-5 ELISPOT. (C) Th1-skewing is shown as a ratio of IFNγ SFC to IL-5 SFC, per mouse. (D) Antigen-specific antibody titers in serum were measured via hemagglutinin inhibition assay. Means and SD are shown. Each symbol represents one mouse, n=6-7 mice per group. Data is representative of two experiments. *p<0.05, ****p<0.0001

CRN-1001 combined with Afluria induced a stronger IFNγ (Th1) influenza-specific T cell response compared to Afluria or Fluad alone. Moreover, the IL-5 (Th2) response was dampened by the addition of R848, resulting in a strong Th1 skew with CRN-1001 compared to Afluria or Fluad alone. Lastly, antibody responses were preserved with the addition of CRN-1001.

CRN-1001 Protects Mice from Lethal PR8 Influenza Challenge

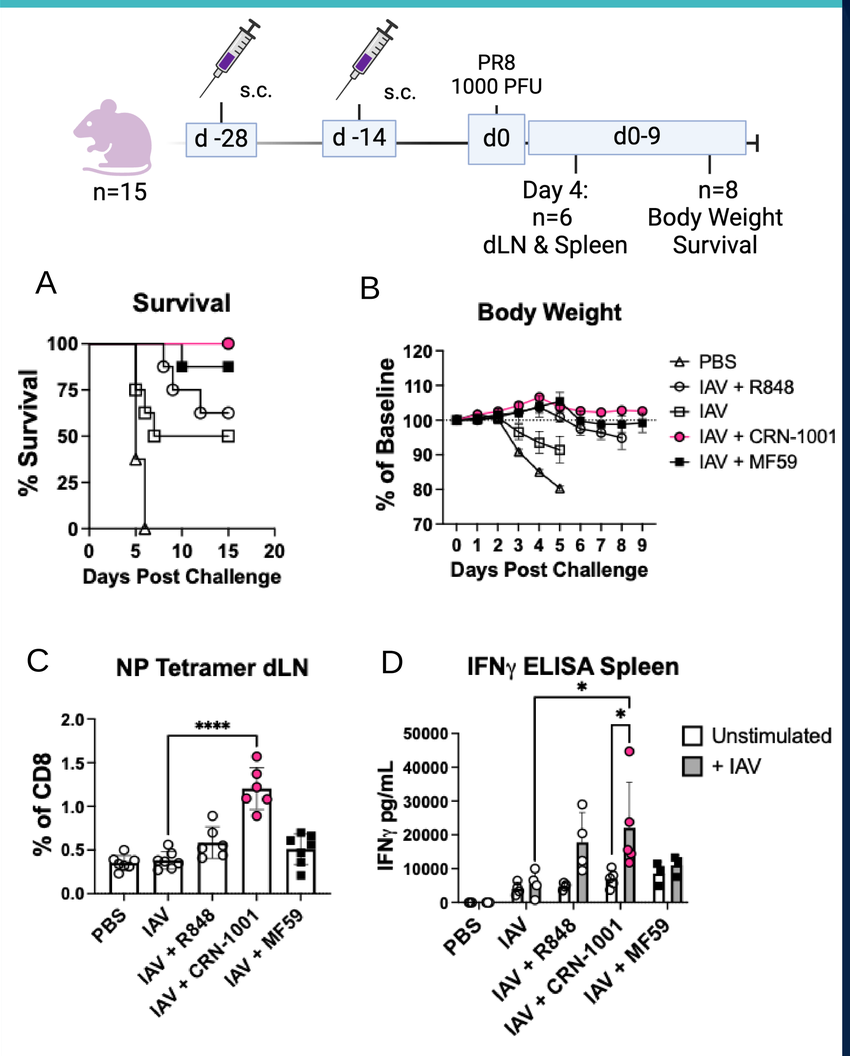


Figure 4. BALB/c mice were immunized s.c with inactivated PR8 influenza virus (IAV) alone or in combination with CRN-1001 or MF59. Mice were infected with a lethal dose of PR8 virus 14 days later. After challenge, one cohort of mice was monitored daily for (A) survival and (B) body weight. Four days after challenge, a cohort of mice was used to assess (C) CD8+ NP tetramer staining in dLN and (D) T cell recall in spleen after restimulation with IAV. Means and SD are shown. n=5-8 mice. Data representative of 2 experiments. *p<0.05, ****p<0.0001

Mice immunized with CRN-1001 and inactivated PR8 influenza virus (IAV) did not develop clinical symptoms or lose body weight and were completely protected after a lethal challenge with live PR8 virus. CRN-1001 enhanced the frequency of influenza nucleoprotein-specific CD8+ cells in draining lymph nodes and induced a stronger PR8-specific IFNγ T cell response in the spleen compared to immunization with IAV alone.

CRN-1001 Enhances Antibody and T Cell Responses Induced by Commercial Influenza Vaccine in Non-Human Primates

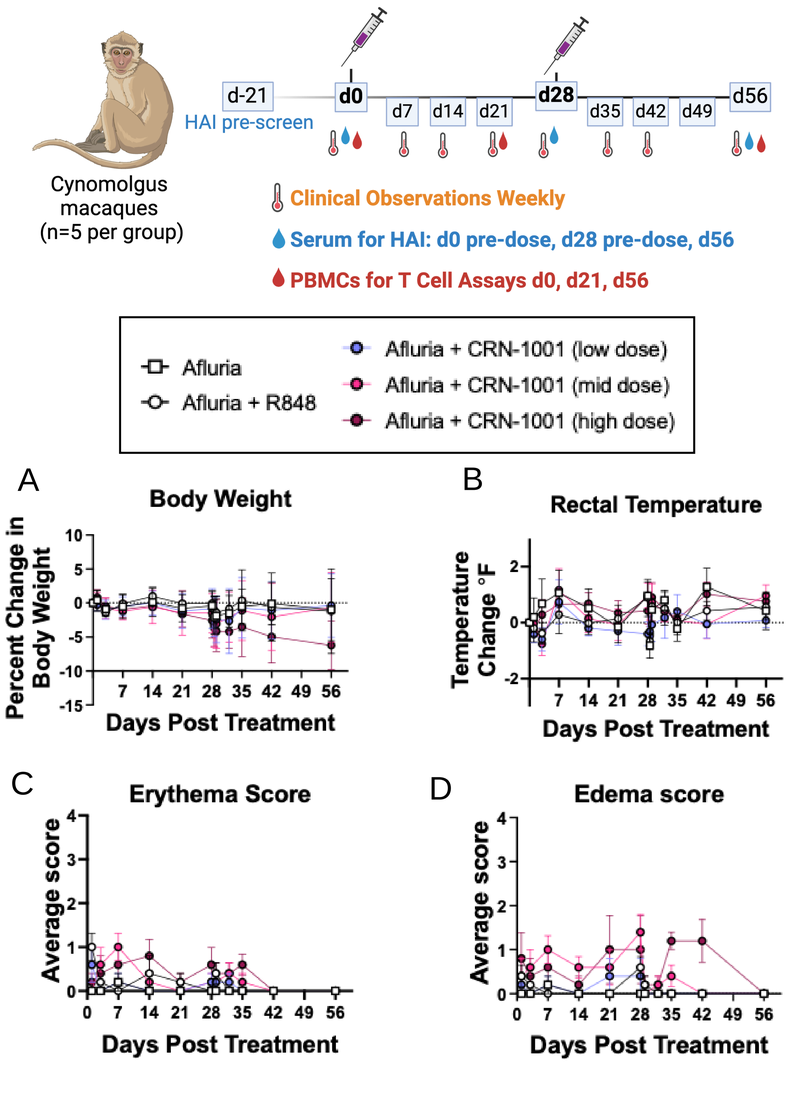


Figure 5: Male and Female Cynomolgus macaques were immunized s.c. with Afluria and various doses of CRN-1001. (A) Body weight, (B) temperature, and (C-D) injection site observations were taken weekly. Serum was taken for assessment of antibody titers against (A) A/Victoria, (B) A/Darwin, (C) B/Phuket, and (D) B/Austria strains via hemagglutinin inhibition assay. PBMCs were used for (I-J) IFNγ ELISPOT with Afluria used as antigen for restimulation. All animals received 30ug Afluria (half of the adult human dose). Means and SD are shown n=5 animals per group.

CRN-1001 was well-tolerated at multiple doses in non-human primates and enhanced the antibody titers against all 4 strains in the Afluria vaccine compared to Afluria alone or Afluria combined with R848. CRN-1001 induced a robust IFNγ T cell response, which was not seen with Afluria alone.

Key Takeaways

- CRN-1001 stimulation results in hyperactive murine BMDCs, which are highly efficient at migrating to dLN and secreting IL-1β while remaining viable
- CRN-1001 enhances the ability of a commercial influenza vaccine to induce antigen-specific IFNγ T cell response while maintaining antibody responses in mice
- CRN-1001 protects against lethal influenza challenge in mice, in part due to enhanced antigen-specific CD8 T cell generation
- CRN-1001 is well-tolerated in non-human primates and enhances both antibody and T cell responses against multiple influenza antigens when combined with a commercially available influenza vaccine