In Vitro Analysis of Synergy between Adjuvants MPLA, CpG, and cGAMP for Treatment of Cancer

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Introduction

Objective: Analyze synergy of adjuvants, such as CpG, cGAMP, and Monophosphoryl lipid A, for Interferon and NF-κβ activation *in vitro,* which would allow for an increase in safety and efficacy of cancer treatments.

Cancer, the uncontrolled growth of cells in the body, is among the most lethal diseases, and the most common form of treatment, chemotherapy, is often unsuccessful or comes with many unwanted side effects. A different form of treatment, immunotherapy, aims to activate the immune system to eradicate the cancer like it would a pathogen. This form of treatment would be more targeted, have fewer side effects, and decrease the chance of recurrence. Utilizing immunotherapeutics, treatments that induce and/or enhance immunological responses, for cancer treatments shows far greater promise in the oncoimmunological field than the application of harmful and toxic chemotherapeutics. Tumor cells can often circumvent detection by the innate immune system; thus, the innate immune system is unable to eliminate the cells.The field of cancer immunotherapy strives to utilize adjuvants to elicit and enhance immunological responses towards cancerous cells. In this study, the synergical application of the codeliverance of three adjuvants, CpG, cGAMP, and monophosphoryl lipid A (MPLA), is examined (Figure 1). By examining the synergy between two adjuvants, immunological responses can be significantly furthered, thereby prompting the body to eradicate the tumor providing a more potent treatment with greater efficacy and safety than traditional chemotherapy.

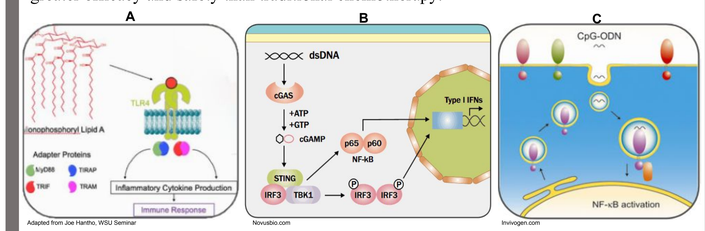


Figure 1. (A) shows a depiction of how the adjuvant, Monophosphoryl Lipid A,is an agonist and modified chemical that signals the TLR4 pathway and initiates an NF-κβ response.[1] (B) is a depiction of cGAMP, a cyclic dinucleotide adjuvant that is an agonist for the STING receptor, activating an interferon response.[2] (C) depicts the ogliodeoxynucleotide and adjuvant CpG, an agonist for the TLR9 pathway, signaling and activating an NF-κβ response.[3]

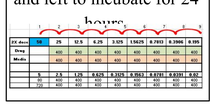
Methods

Seeding

RAW Dual reporter cells, mouse macrophages, were counted, and diluted to obtain a 200,000 cells/mL concentration. The suspended cell solution was plated at 20,000 cells/well and incubated overnight

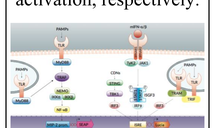
Dosing

Cells were dosed with a combination of adjuvants: cGAMP and MPLA, CpG and cGAMP, and CpG and MPLA. Cells were dosed at the appropriate ratio and concentration and left to incubate for 24 hours.



Analyzation

Quantiblue and quantiluc assays were performed on cell supernatant to quantify the amount of NF-κβ and interferon activation, respectively.



The CellTiter-Glo assay was used to determine if any cell death occured.

Results

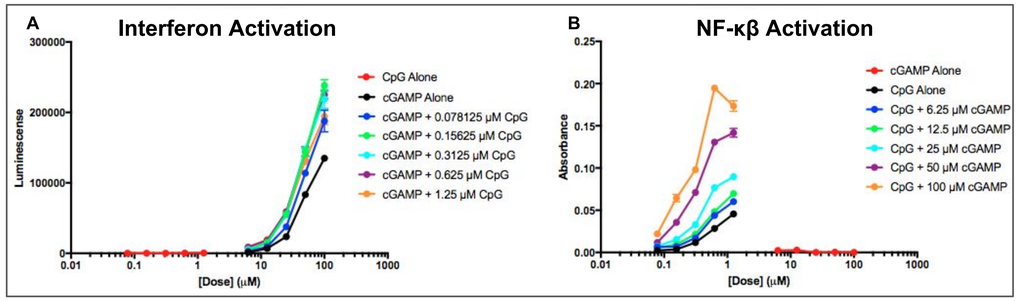


Figure 2. Examination of synergy between cGAMP and CpG. (A) Interferon response of doses of cGAMP between 6.25 μM and 100 μM with added fixed concentrations of CpG between 0.078125 μM and 1.25 μM was measured. The interferon response of the two adjuvants, cGAMP and CpG, was measured alone to provide a baseline. CpG alone does not elicit an interferon response; however, when the fixed doses of CpG are added to cGAMP, there is a greater interferon response. **(B)** NF-κβ response of doses of CpG between 0.078125 μM and 1.25 μM with added fixed concentrations of cGAMP between 6.25 μM and 100 μM was measured. The NF-κβ response of the two adjuvants, CpG and cGAMP was measured alone to provide a baseline. cGAMP alone does not elicit an NF-κβ response; however, when the fixed doses of cGAMP are added to CpG, there is a greater NF-κβ response.

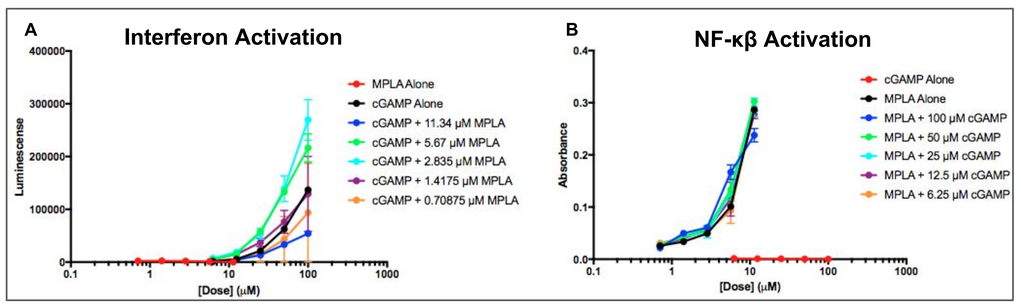


Figure 3. Examination of synergy between cGAMP and MPLA. (A) Interferon response of doses of cGAMP between 6.25 μM and 100 μM with added fixed concentrations of MPLA between 0.70875 μM and 11.34 μM was measured. The interferon response of the two adjuvants, cGAMP and MPLA, was measured alone to provide a baseline. MPLA alone does not elicit an interferon response; however, when the fixed doses of MPLA are added to cGAMP, there is a greater interferon response. **(B)** NF-κβ response of doses of MPLA between 0.70875 μM and 11.34 μM with added fixed concentrations of cGAMP between 6.25 μM and 100 μM was measured. The NF-κβ response of the two adjuvants, MPLA and cGAMP was measured alone to provide a baseline. There is no enhanced activation when cGAMP is added MPLA doses.

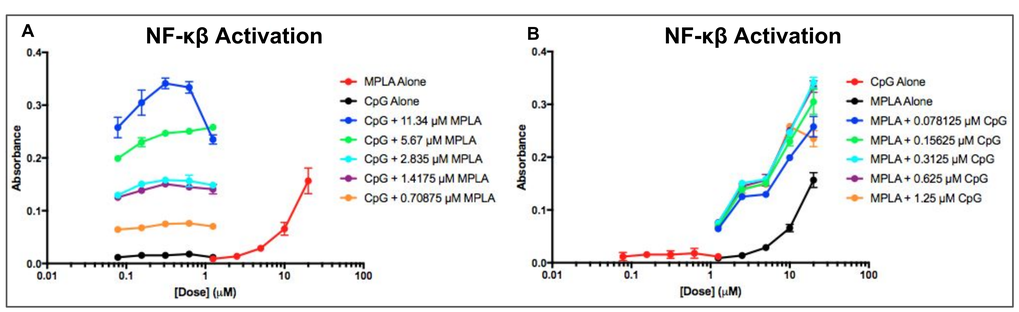


Figure 3. Examination of synergy between CpG and MPLA. (A) NF-κβ response of doses CpG between 0.078125 μM and 1.25 μM with added fixed concentrations of MPLA between 0.70875 μM and 11.34 μM was measured. The NF-κβ response of the two adjuvants, CpG and MPLA, was measured alone to provide a baseline. MPLA alone has NF-κβ response at higher concentrations and CpG alone has minimal NF-κβ response. When given in combination, there is a higher response than with either adjuvant alone. **(B)** NF-κβ response of doses of MPLA between 0.70875 μM and 11.34 μM with added fixed concentrations of CpG between 0.078125 μM and 1.25 μM was measured. The NF-κβ response of the two adjuvants, MPLA and CpG was measured alone to provide a baseline. At all concentrations, CpG enhances the NF-κβ response caused by MPLA.

Discussion

- Figure 2 shows that cGAMP alone provided an interferon response, but when CpG was added, response was enhanced at all doses, even though CpG alone caused no interferon response. CpG alone elicited an NF-κβ response, but when cGAMP is added to CpG, NF-κβ response is enhanced depending on the concentration of the added cGAMP.
- Figure 3 shows that when MPLA is added to cGAMP, interferon response is enhanced when higher doses of the MPLA are added. The NF-κβ graph shown in Figure 3B shows that NF-κβ response is not enhanced when cGAMP is added to MPLA.
- Figure 4 shows that when MPLA is added to CpG, NF-κβ response is enhanced when higher concentrations of MPLA are added to CpG. Graph B depicts that when CpG is added to doses of MPLA, NF-κβ activation is enhanced independent of the added CpG doses.
- Overall, several adjuvants combinations show preliminary synergy, which may be enhanced by codelivery

Future Directions

- Continuation of the analysis of synergy to find precise ratios that will, when delivered *in vivo,* yield the greatest amount of immunological response, the most tumor cell death, and the least off-target cell death, at more efficient and lower concentrations of drug
- Utilization of polymersomes to encapsulate the adjuvants for better delivery into cells
- Co-encapsulate drugs rather than delivering drugs without polymersomes or encapsulating drugs into different polymersomes in order to ensure exact ratio enters each cell
- Utilize polymersomes with multiple adjuvants to treat *in vivo* models of cancer in mice
- Utilize synergy results to vaccines with multiple adjuvants that would be used to treat cancer *in vivo* in mice because there would be a stronger innate immune response

Acknowledgements

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