In Vivo Evaluation of CpG DNA Complexed with Calcium Phosphate as a Vaccine Adjuvant

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Primary Sources of 2019 NNCI iREU Funding: National Science Foundation via awards ECCS-1626153 (NNCI Coordinating Office at Georgia Tech) and NNCI-1542081 (Cornell NanoScale Facility)

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Abstract:

One of the key biomedical developments of the 20th century was massive growth in the development and deployment of vaccines. One element in increasing the effectiveness of vaccines was the discovery of certain substances that could be used as vaccine adjuvants, improving the immunostimulatory effects of the vaccine. Recently, a new type of adjuvant, referred to as CpG DNA, has shown promise as a basis for a next generation vaccine adjuvant. Complexing this CpG DNA (K3) with calcium phosphate (CaP) nanoparticles leads to an increase in both ThI and ThII-type humoral immunities, as well as a generation of cellular immunity. Since CaP has an adjuvant effect in and of itself, our main question is whether K3 complexed with CaP is a more effective adjuvant than K3 mixed with CaP. To test this, we inoculated murine models with OVA cancer vaccine under four conditions: no adjuvant, K3, K3 complexed with CaP (K3/CaP), and K3 mixed with CaP (K3+CaP). The immune responses were determined by sampling blood and analyzing with FACS and ELISA. The results indicated that while K3/CaP and K3+CaP produce similar humoral immunity responses, K3/CaP is a more effective vaccine adjuvant for the purpose of promoting cellular immunity.

Summary of Research:

CpG DNA adjuvants are composed of a short DNA sequence that contains a cytosine-guanine motif that frequently occurs in pathogen DNA but rarely in human DNA. One of the most interesting CpG DNA adjuvants is called K3, with the sequence 5'-at**cg**actct**cg**ag**cg**ttctc-3'. This adjuvant has been shown not only to induce ThI-type immunity, which results in increased levels of IgG2c antibodies, but also to induce cellular immunity resulting from the generation of cytotoxic CD8+ T cells that are capable of killing infected cells. Recent efforts to improve K3 as an adjuvant have included complexing it with calcium phosphate (CaP) nanoparticles. The complex not only affords more efficient delivery of the K3 DNA to the target cells, but also, because CaP is itself an adjuvant, induces ThII-type immunity (increased levels of IgG1 antibodies) in addition to the ThI-type and cellular immunities ordinarily generated by K3.

The adjuvant effects of naked CaP raise an interesting question: might an adjuvant composed of K3 mixed with CaP (K3+CaP) be more efficient than one composed of K3 complexed with CaP (K3/CaP)? The purpose of this study is to explore this question. The results of this study ought to bring K3-based adjuvants a step closer to large-scale deployment.

Oligodeoxynucleotides (ODNs) were obtained and prepared by the methods used by Hanagata, et al. [1]. ODN-CaP complexes were prepared by an adsorption method [1]. For the cancer cells, a mouse cancer line, E.G7, was used. Mice were obtained and immunized following the procedure described by Hanagata, et al. [1]. Specifically, mice were immunized with a dose of vaccine (either ovalbumin [OVA] only, OVA + K3, OVA + K3/CaP, or OVA + K3+CaP) on day one, and again with the same vaccine one week later. After this, nine days were allowed to elapse before transplanting EG7 cells into the mice. Eleven days after transplantation, any developed tumors were excised, and blood was sampled for characterization. Characterization of

the mouse blood was conducted using fluorescence-activated cell sorting (FACS) for CD8+ cell counts and enzyme-linked immunosorbent assays (ELISA) for quantification of IgG1 and IgG2c levels.

Results from ELISA are illustrated in Figures 1 and 2. As can be seen, though both K3/CaP and K3+CaP induced greater amounts of IgG1 and IgG2c relative to the control and K3 groups, there is no statistically significant difference between the relative amounts of IgG1 or IgG2c generated between K3/CaP and K3+CaP. Results from FACS are illustrated in Figures 3 and 4. Again, both K3/CaP and K3+CaP resulted in higher relative amounts of CD8+ T cells than did the control group. However, while the K3+CaP group resulted in amounts of CD8+ T cells generated similar to K3, the K3/CaP group had amounts of CD8+ T cells significantly higher than K3 and K3+CaP. Taken together, this indicates that, while K3+CaP and K3/CaP are similarly effective at inducing humoral immunity and are both more effective than K3 in that regard, K3/CaP is the most effective adjuvant for inducing humoral and cellular immunity simultaneously.

Acknowledgements:

I would like to thank Dr. Nobutaka Hanagata at the National Institute for Materials Science (NIMS) for hosting me this summer, as well as Dr. Xianglan Li for working closely with me over the course of the summer and the staff of NIMS for providing additional support. I would also like to thank Dr. Lynn Rathbun for his work coordinating this program. Finally, I would like to thank Cornell University and the National Science Foundation for providing funding for this summer via awards ECCS-1626153 (NNCI Coordinating Office at Georgia Tech) and ECCS-154208 (Cornell NanoScale Facility).

References:

[1] Hanagata N, Li X, Chen M, Li J, Hattori S. Double-stranded phosphodiester cytosine-guanine oligodeoxynucleotide complexed with calcium phosphate as a potent vaccine adjuvant for activating cellular and ThI-type humoral immunities. International Journal of Nanomedicine. 2018; 13: 43-62.

[2] Hanagata N. CpG oligodeoxynucleotide nanomedicines for the prophylaxis or treatment of cancers, infectious diseases, and allergies. International Journal of Nanomedicine. 2017; 12: 515-531.

[3] Takahashi H, Misato K, Aoshi T, Yamamoto Y, Kubota Y, Wu X, Kuroda E, Ishii KJ, Yamamoto H, Yoshioka Y. Carbonate apatite nanoparticles act as potent vaccine adjuvant delivery vehicles by enhancing cytokine production induced by encapsulated cytosine-phosphate-guanine oligodeoxynucleotides. Frontiers in Immunology. 2018; 9: 1-17.

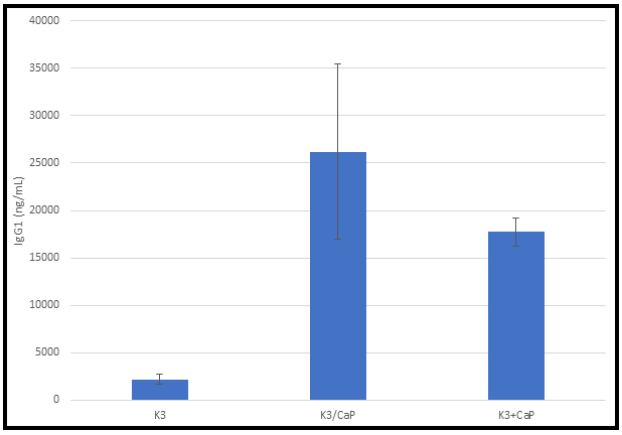

Figure 1: ELISA, IgG1.

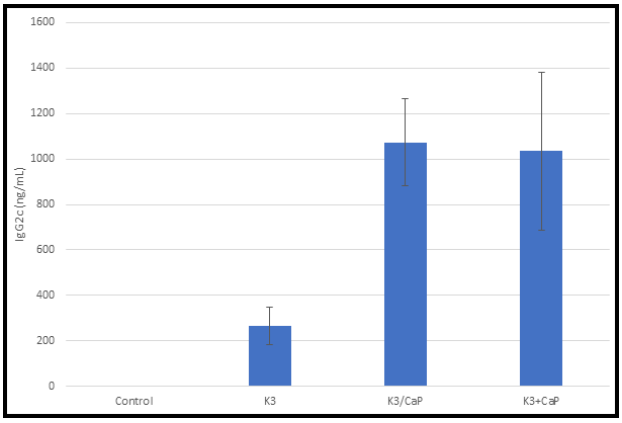

Figure 2: ELISA, IgG2c.

K3/CaP	1	2	3	4
C-Q1	0.45%	0.39%	0.50%	0.38%
C-Q2	1.30%	2.24%	2.96%	3.76%
C-Q3	63.42%	62.79%	61.77%	56.15%
C-Q4	34.83%	34.58%	34.77%	39.71%

Figure 3: FACS, K3/CaP

K3+CaP	1	2	3	4
C-Q1	0.38%	0.33%	0.38%	0.42%
C-Q2	0.90%	1.91%	2.48%	1.11%
C-Q3	59.57%	59.91%	59.22%	62.26%
C-Q4	39.15%	37.85%	37.92%	36.21%

Figure 4: FACS, K3+CaP