Regulation of the Immune System by DNA-Drug Nanomaterials

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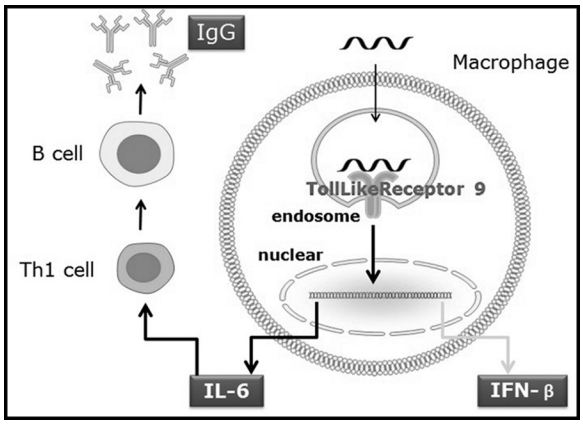
Abstract:

Single stranded cytosine-phosphate-guanine ogliodeoxynucleotides (ss-CpG ODN) have been shown to bind to Toll-like receptor 9 (TLR9) located in the endosome of macrophagesin the immune system. This allows for regulation of both the innate and adaptive immune system that can lead to medical treatments such as cancer immunotherapy. Double stranded non-CpG ODN (ds-non-CpG ODN) are capable of regulating the innate immune system through interactions with cytosolic receptors. Our goal for this project was to investigate how the shape of different nanomaterials can affect the action of the ODN drug in macrophages. Both ss-CpG ODN and ds-non-CpG ODN were functionalized separately onto a cationic lipid DOTAP, carbon nanohorn (CNH), polyethyleneimide-coated CNH, and MoS_2 monolayer sheet. The ODN-nanomaterial solutions were transfected to macrophages and the RNA was isolated. Finally, reverse transcription and real time polymerase chain reaction were performed to measure the relative expression level of interleukin 6 (IL-6) and interferon beta (IFN-β), two proteins secreted in the adaptive and innate immune system pathways respectively. It was found that for both ss-CpG ODN and ds-non-CpG ODN, samples incubated with DOTAP had the highest level of expression IL-6 and IFN-β.

Introduction:

Ss-CpG ODN and ds-non-CpG ODN are both capable of regulating the immune system through different pathways (Figures 1 and 2). Ss-CpG ODN activates the adaptive immune system through the TLR9 in the endosome of macrophages of the immune system, which in turns produces the protein IL-6. Ds-non-CpG ODN activates the innate immune system through binding different cytosolic DNA receptors in the cytosol that produces the protein IFN-β. For this project, our goal was to investigate how the shape of the nanomaterial can affect the action of the DNA drug in macrophages. DOTAP is a cationic lipid that composes the membrane around the endosome in macrophages, which is capable of binding the drug with electrostatical interaction. Molybdenum disulfide (MoS_2) sheets are monolayer sheets similar in morphology to graphene.

Finally, CNH are similar to single walled carbon nanotubes but are around 10 to 20 nm and form a cone shape. They aggregate together to form particles around 60 nm long. For both MoS_2 and CNH, the drug is adhered to the surface of the nanomaterial. Because CNH and the ODN are both negatively charged, it is unclear what forces are adsorbing the ODN onto the surface of the CNH. Ss-CpG ODN is believed to change conformation when adhered to the nanomaterial, which in turn can affect how it interacts with TLR9, leading to either enhanced or lower the immune response. For ds-non-CpG ODN, the nanomaterial acts as a carrier for the ODN drug out of the endosome and into the cytosol.



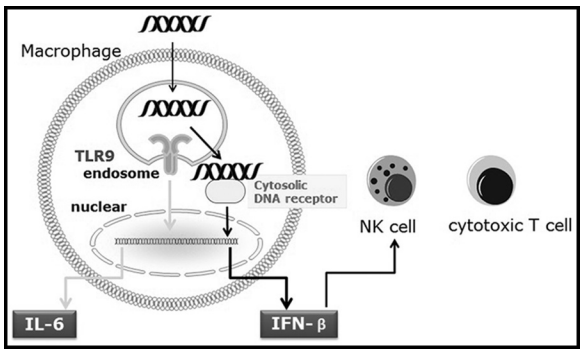


Figure 1, top: Diagram of the immune system activation pathway for ss-CpG ODN. Figure 2, bottom: Diagram of the immune system activation pathway for ds-non-CpG ODN.

Experimental Procedure:

To see if the nanomaterial drugs are activating the immune system, we measured the levels of IL-6 and IFN-β. First, we functionalized the ODN onto the nanomaterial and added it to a culture of macrophages, allowing it to incubate overnight. After loading the DNA onto the nanomaterial, except for the case of DOTAP, the material was ultracententrifuged and the supernatant collected and measured using ultra-violet/visible light spectroscopy. From this, the mass amount of DNA loaded onto the material is calculated. The ribonucleic acid (RNA) from the cell was then isolated and cleaned up to remove impurities.

Next, reverse transcription is performed to transcribe the RNA back to deoxyribonucleic acid (DNA). The amount of protein produced in the cells is inferred from the amount of DNA measured using real-time polymerase chain reaction (q-PCR). The cellular DNA is mixed with buffer and primer that is complement to the sequence of DNA for either IL-6 or IFN-β. GAPDH, a house-keeping protein that has a stable production in cells throughout different conditions, was used as a housekeeping protein to standardize the expression level against the other samples.

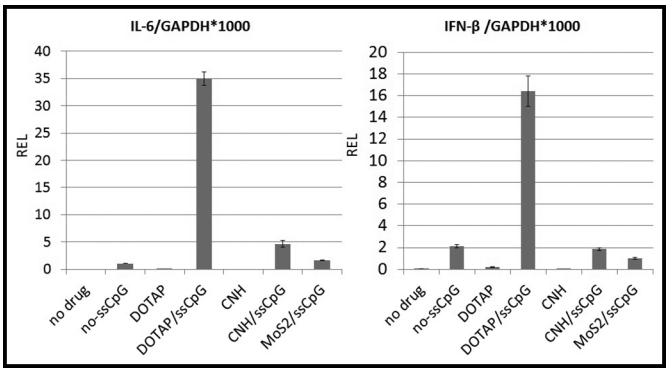


Figure 3: Relative expression level of IL-6 (a) and IFN-β (b) for ss-CpG ODN with various nanomaterials.

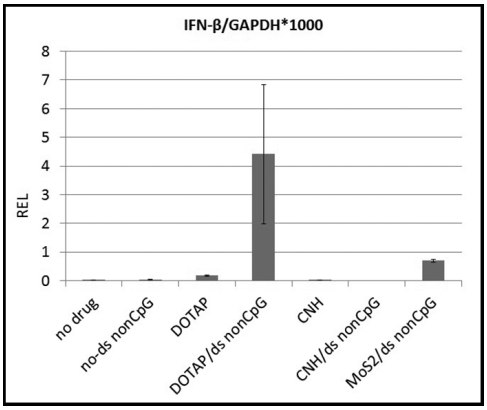


Figure 4: Relative expression level IFN-β for ds-non-CpG ODN with various nanomaterials.

Results and Conclusions:

The results of IL-6 and IFN-β for nanomaterials loaded with ss-CpG ODN are shown in Figure 3. We observed that DOTAP was the most effective nanomaterial for delivery of ss-CpG ODN to the TLR9 in the endosome. From this result, we can conclude that the ODN adsorbed onto the surface of the other nanomaterials is not able to interact with TLR9. For the ds-non-CpG ODN, only the production of IFN-β was investigated. Consequently, DOTAP also had the highest potential to induce IFN-β for the delivery of ds-non-CpG ODN (Figure 4). The biggest challenge with activating the cytosolic receptors is that the DNA must leave the endosome. Because DOTAP is composed of the same material that the endosome membrane is composed of, it can combine with the membrane, releasing the loaded drug into the cytosol while the other materials must diffuse through the membrane to deliver to the cytosolic receptors.

Future Work:

Due to the large error in the ds-non-CpG ODN experiment, more trials would need to be completed to verify the work. To investigate the effects of electrostatic interaction versus adsorption on the ODN, the same experimental steps seen here could be performed on CNH sample but coated in polyethylene imide (PEI), a positively-charged polymer, before ODN functionalization.

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