A potential new treatment for Multiple Sclerosis

DNA nanoparticles stimulate Interferon Beta production in dendritic cells

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Introduction:

Multiple Sclerosis (MS) is a disease in which the immune system attacks the body's own nerve cells. There is currently no cure for MS, however the symptoms, including pain and loss of mobility, can be treated using a protein called interferon, which is usually produced by the immune system in response to a viral infection. However DNA nanoparticles can also stimulate interferon production in the cell. This project aimed to find which type of nanoparticle produces the best interferon response, and therefore would be most likely to show success as a treatment for MS.

Objectives:

To test different polymers, including peptide LAH4, for their ability to form a nanoparticle with DNA and to subsequently stimulate interferon production in dendritic cells.

Methods:

- To establish the HPLC method to examine the activity of the enzyme IDO, which is induced by type I interferons in the STING pathway.
- Expose dendritic cells to DNA nanoparticles made using various different polymers, including PEI and LAH4, to see how well the nanoparticle is taken up by the cells and whether interferon beta is produced as a result. This was determined by extracting the RNA from the cells then using the RNA to perform real-time quantitative PCR to test for the presence of interferon beta.
- Determine which polymer works best as part of a DNA nanoparticle then optimise the NP ratio to form the most efficient nanoparticle.

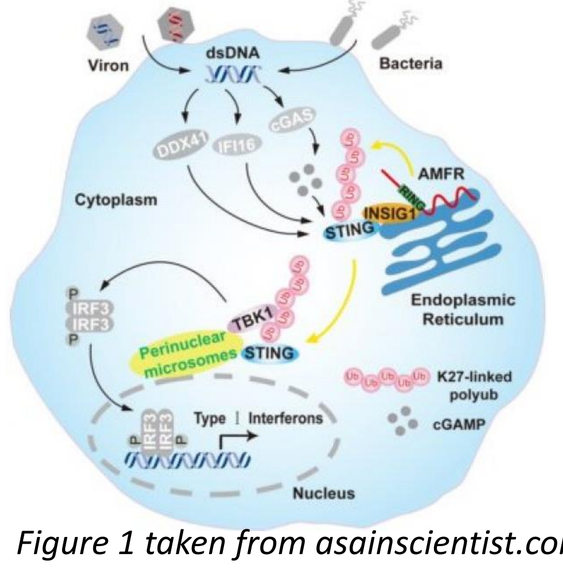
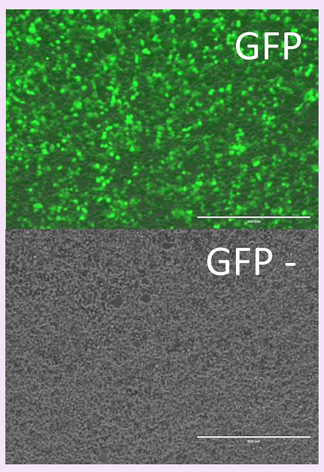
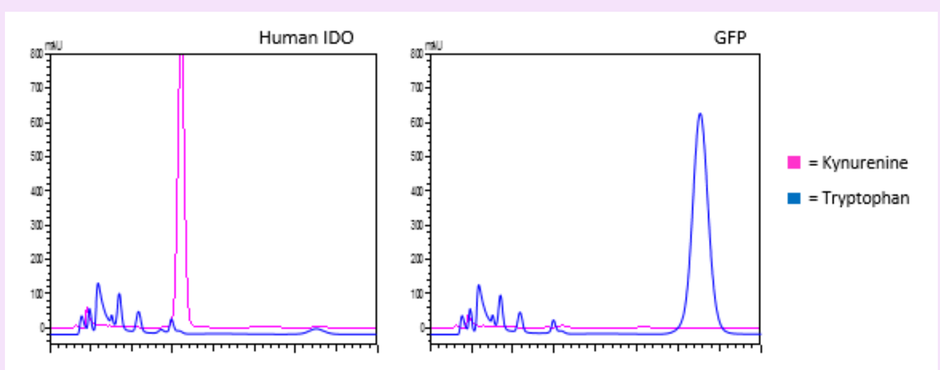


Figure 1 taken from asainscientist.com

Results:

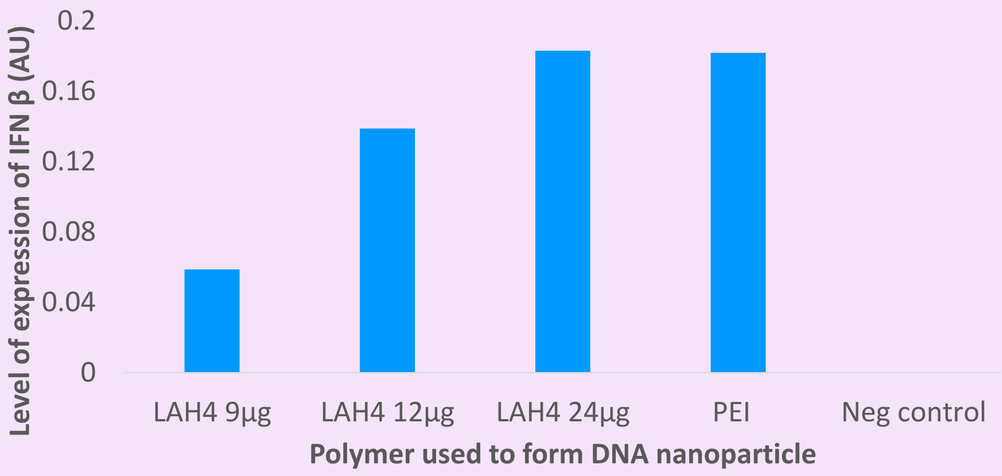
These images were taken of cells, successfully transfected with green fluorescent protein (GFP), fluorescing under green light and non transfected cells showing no fluorescence. Once the GFP was shown to have been successfully transfected into the cells then plasmids containing IDO and GFP could be transfected into dendritic cells, with GFP showing positive uptake.





These HPLC (high performance liquid chromatography) traces show levels of kynurenine and tryptophan detected in the cells transfected with human IDO and GFP.

IDO is an enzyme which converts tryptophan into kynurenine, therefore when IDO is present kynurenine levels should be high and tryptophan should be low, and vice versa when there is no IDO only GFP. The HPLC results show this trend was indeed observed in our transfected cells, showing IDO was present.



This bar graph shows how well different polymers were able to form a nanoparticle able to induce interferon beta in dendritic cells. Different concentrations of the peptide LAH4 were tested, along with the positive control PEI and the negative control. 24µg of LAH4 caused the highest level of expression of IFN β, even higher than PEI.

Conclusions:

The results from this project suggest that LAH4 is the most efficient polymer to use to form a DNA nanoparticle. The efficiency of each of the nanoparticles was assessed based on their ability to enter the cell without causing it to burst and die, and then on its ability to cause increased interferon beta levels in the cell. Based on the results from the quantitative PCR the LAH4 nanoparticles produced the highest levels of interferon beta compared to other polymers such as PLL and PLH, and similar levels to PEI. 24µg of LAH4 combined with 4µg produced the best performing nanoparticle, as shown in the bar graph above.
Therefore LAH4 showed the most promise as a potential future treatment for Multiple Sclerosis.

Future work:

The next step will be to test the safety of the LAH4 DNA nanoparticle in a healthy animal model, and if that is successful then different doses can be tested on a MS animal model to see if there is any improvement in the symptoms of the disease.

Acknowledgements:
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Acronyms:
- IFN β = Interferon Beta
- GFP = Green fluorescent protein
- HPLC = High performance liquid chromatography
- STING = Stimulator of interferon genes

References:

Figure 1 taken from: Ubiquitination The Missing Link In The STING Pathway. Asian Scientist Magazine. https://www.asianscientist.com/2015/01/in-the-lab/ubiquitination-missing-link-sting-pathway/ [Accessed 05/10/2017]