**////Title: Improving Vaccine Protection for the Elderly**

**////Stand-first:**

The greatest challenge for ageing populations is that vaccines can be less protective for the elderly due to the age-related decline of the immune system. This means that improving the efficacy of vaccines in the ageing population is crucial to public health. Dr Lei Jin [lā] [jin] and colleagues from the University of Florida set out to develop a novel strategy to directly address this key issue.

**////Body text:**

The number of people aged 65 years or over is projected to more than double in the next three decades, reaching over 1.5 billion in 2050. The ongoing COVID-19 pandemic has shown us just how vulnerable ageing populations are to emerging pathogens and has proven that vaccination offers an incredibly efficient and cost-effective method to protect the most vulnerable people across the globe.

Vaccines teach your body how to destroy infectious pathogens, such as bacteria and viruses, by introducing a harmless part of the pathogen to your immune system. The main aim of a vaccine is to stimulate a specific immune response that will provide long-lasting protection.

As we age, our immune systems become less able to respond effectively to vaccination. The age-related decline of your body’s immune response is known as immunosenescence. Current strategies to counteract immunosenescence include giving a higher antigen dose, using an alternative route of immunisation and employing the use of adjuvants.

Adjuvants are substances added to vaccines which make your body’s immune response more effective. Using adjuvants in vaccines can lead to greater long-term protection from disease when you encounter the same pathogen in the future.

Dr Lei Jin and colleagues at the University of Florida studied a particular adjuvant called cyclic di-GMP, or CDG, in mice. Their aim was to develop a strategy that could enhance the ability of the CDG adjuvant in the aged and ultimately improve vaccine protection for the elderly.

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Cyclic di-GMP is a type of cyclic dinucleotide (or CDN) adjuvant. Essentially, it is a molecule borrowed from bacteria which can stimulate our immune systems by activating specific defence mechanisms that lead to a long-lasting protective immune response. Vaccine-grade cyclic dinucleotides are safe in animal studies and human clinical trials. So far, most studies on CDN adjuvants have been carried out in young mice and an important question to be answered about CDN adjuvants, like CDG, is how their activity declines with age.

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Dr Jin and colleagues first set out to understand how cyclic di-GMP or CDG becomes a less effective adjuvant with age by studying CDG as an adjuvant for a flu vaccine given to adult and aged mice.

First, they gave the mice a vaccine which contained the CDG adjuvant and the Influenza A virus nucleoprotein, a protein that makes up a structural part of the virus. The mice were vaccinated twice every two weeks and the levels of antibodies for the virus nucleoprotein were measured from blood serum and from the lungs 30 and 60 days after vaccination.

Immunoglobulin G antibodies are the most abundant type of antibodies in the blood and Immunoglobulin A are the most abundant antibodies in the lung. Both Immunoglobulin G and Immunoglobulin A antibodies are important for robust immune protection, and the production of Immunoglobulin A is particularly important in the lungs to stop respiratory infections and prevent disease transmission.

After measuring levels of Immunoglobulin G and Immunoglobulin A antibodies in adult and aged mice, the researchers found that aged mice had a significant reduction in antibody levels of Immunoglobulin G in their serum and a reduction in Immunoglobulin A antibodies in the lungs, suggesting that the action of the CDG adjuvant is impaired in aged mice.

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Next, they investigated the ability of aged and adult mice to generate particular immune responses after vaccination with the Influenza A and CDG vaccine. Vaccine-induced Th1 immune responses are necessary for protection from intracellular pathogens, Th17 responses are important for fighting bacterial infections and Th2 responses are important for fighting extracellular pathogens and bacteria.

After measuring the responses post-vaccination, they found significantly reduced Th1, Th2 and Th17 immune responses in blood serum and the lungs of aged mice (similar to those of a 65-year-old human). Together, these results showed that CDG is less effective as an adjuvant when vaccinating the aged mice.

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The next question Dr Jin and colleagues sought to answer was exactly how the CDG adjuvant becomes less effective in aged mice. One of the modes of action of the CDG adjuvant is to specifically activate dendritic cells in the lungs.

Dendritic cells are immune cells that take a piece of a pathogen and present it on their surface to other immune cells. Dendritic cells are important mediators of immune responses and two types of these cells, known as cDC2 and moDCs, were discovered to be involved in the activity of CDG as an adjuvant.

By administering CDG to the aged and adult mice and measuring the number of these particular dendritic cells, they found that aged mice had fewer activated cDC2 cells that are critical for CDG adjuvanticity.

After CDG activates the cDC2 cells, these cells then produce tumour necrosis factor. Tumour necrosis factor is a protein with many roles in the immune response and the production of this protein in the lungs was discovered to be crucial for CDG to work as an adjuvant.

Dr Jin and colleagues next measured the levels of lung tumour necrosis factor and noticed that aged mice had elevated levels of basal tumour necrosis factor in the lungs compared to the adult mice. They found that administering CDG directly into the lungs did not lead to the production of more tumour necrosis factor protein in the aged mice. Importantly, this suggests that the cDC2 cells in aged mice do not respond to the CDG adjuvant in the same way as the adult mice do.

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cDC2 activates moDCs to mediate CDG adjuvant activity in the lung. They found that after administering CDG there were fewer activated moDCs in the aged mice than in the adult mice. This suggested that the activation of the moDC is impaired in aged mice.

The researchers then asked at what age the activity of the CDG adjuvant starts to decline. This time, they ran the same studies on 1-year-old mice, the equivalent of middle-aged humans around 42 years old, and found that both the immune memory responses and the high level of specific antibody production that were impaired in aged mice were also impaired in the 1-year-old mice.

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Finally, after discovering that effectiveness of CDG as an adjuvant is dependent on age, Dr Jin and colleagues set out to design a protein that could enhance the ability of the CDG adjuvant in the aged. Based on their observations that the moDC cells are less able to become activated by CDG in aged mice, they suggested that directly activating more moDCs could counteract this process in the aged.

In addition, the observation that aged mice are not as able to produce tumour necrosis factor in the lungs when administered with CDG lead the authors to hypothesise that if they supplemented the moDC targeting with tumour necrosis factor, they may be able to improve the effectiveness of CDG in aged mice.

Consequently, they designed two fusion proteins containing tumour necrosis factor which can activate moDCs. They then vaccinated both middle-aged and aged mice with a vaccine that included the CDG adjuvant and the fusion protein.

They found that the fusion proteins they had developed were able to successfully restore the effectiveness of the CDG adjuvant in both middle-aged and aged mice. By adding the fusion proteins to their vaccines, they were able to restore the Th1 and Th17 responses that were depleted in aged mice whilst increasing specific antibody production. Importantly, this included a significant increase in specific Immunoglobulin A production in the lungs.

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The observation that CDG adjuvanticity depends on age is an important discovery for the design of future CDN-based vaccines for the elderly. With an increasingly ageing population, the ability of the new moDC targeting strategy, developed by Dr Jin and colleagues introduces a promising new strategy to circumvent the consequences of immunosenescence to vaccine protection of the elderly.

This SciPod is a summary of the paper ‘New MoDC-Targeting TNF Fusion Proteins Enhance Cyclic Di-GMP Vaccine Adjuvanticity in Middle-Aged and Aged Mice’, from Frontiers in Immunology. DOI: <https://doi.org/10.3389/fimmu.2020.01674>.

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