Adjuvants and Their Role in Vaccines

Dr. Schabort Froneman, Zoetis South Africa (Pty) Ltd, Technical Manager: Ruminants

Immunology is a fascinating subject and vaccination is one of the most successful ways of preventing disease in both humans and animals.[1] The World Health Organisation (WHO) states that vaccination prevented 10 million (human) deaths between 2010 – 2015 and protected many additional millions of people from illness.[2] The public health benefits of vaccination (of humans and animals alike) could hardly be overstated and the recent COVID 19 outbreak underlined the devastating effects of infectious disease in an immunologically naïve population.

The Chinese used inoculation techniques to produce immunity against smallpox as early as 900 AD.[3] Inoculation is the method of transferring the actual disease-causing agent and thus inducing the disease and after recovery the individual would then be immune against the disease. This practice unfortunately resulted in many cases of severe disease and even mortalities.
Edward Jenner is the first scientist to effectively develop vaccination, in that he induced immunity against smallpox in human patients that were experimentally infected with cow pox, the latter causing only mild clinical symptoms.[3]

Since that first discovery, the scientific and medical world spent a lot of effort in the development of commercial vaccines. Early vaccines were generally live-attenuated or whole-pathogen preparations. Although some of these preparations are still being used today, concerns for safety and potential to cause disease, have directed the research towards subunit and inactivated vaccines.[3] This is especially true in human vaccines and less so in animal vaccines.

Inactivated and sub-unit vaccines are generally less immunogenic than their modified live counterparts and benefit greatly from the use of adjuvants to ensure that adequate immunity develops upon administration.[4] This being said, the benefits of adjuvantation in modified live vaccines cannot be overlooked as they have the ability to enhance viral antigen immunogenicity. This is not to say that they do not have important benefits in modified live vaccines as well, especially if they have the ability to increase viral antigen immunogenicity.

The word “Adjuvant” is derived from the Latin word *adjuvare*, meaning “to help”.[5] Adjuvants are substances that, when given together with an antigen, enhances the rate, magnitude, format or quality of the immune response to that antigen.[6]

Adjuvants have been improving vaccines for as long as vaccines have been used, for example the live viral inoculation of the early Chinese contained intrinsic adjuvants in the form of viral nucleic acid, the viral coat and possibly bacterial contamination.[7]
The history of adjuvant discovery, however, starts with a French veterinarian named Gaston Ramon. While working at the Pasteur Institute in 1920, Ramon discovered that higher specific antibody titres were detected in horses that developed an abscess at the injection site.[1] He then replicated these results by inducing sterile abscesses (that resulted in inflammation at the injection site) with various substrates such as starch, breadcrumbs or tapioca. By significantly increasing antibody production with the addition of these non-specific inflammatory stimulants, he effectively discovered adjuvants.[6] In the same time-period, Alexander Glenny et al. discovered the immune enhancing effects of aluminium salts.[1]

Aluminium salts were added to vaccines in 1932 and were the only licenced adjuvant in human vaccines for the following 70 years. Aluminium compounds still form the basis for most human vaccines.[6]

Following the implementation of aluminium salt adjuvants, oil emulsions were shown to express highly effective adjuvant properties. Freund's adjuvant is widely used in research and are formulated as *Freund Incomplete Adjuvant (FIA)*, a water-in-mineral oil emulsion or *Freund Complete Adjuvant (FCA)* that also contains heat killed mycobacteria. Although potent, Freund's adjuvant is too reactogenic (with potential to cause injection site granulomas, abscesses or necrosis) to be utilised in commercial vaccines.[1, 6]

These scientists laid the foundational groundwork for adjuvant research and development, providing us with the variety of commercial adjuvants available today, as well as exciting research for future formulations. Although the bulk of vaccinology research goes into antigen determination and the development of new vaccines, adjuvant research is growing as scientists realise the important ro1le they play in potentiating mammalian immune responses and subsequently enhancing vaccine efficacy.

The last two decades have seen a lot of progress being made in the understanding of the mechanism of action of adjuvants, but a lot remains unclear and efficacy of these substances are often empirically derived.

In general, adjuvants augment the immune response in one of the following ways[5, 6]:

1. Regulating antigen release to prolong persistence
2. Enhanced response to antigen exposure
3. Regulation of the quality of the immune response

EXAMPLES OF ADJUVANTS THEIR MECHANISMS OF ACTION[1, 4, 6, 7]:

Aluminium salts are the first licenced adjuvants used and have a long-standing safety record. Their ease of formulation and ability to induce high antibody titres with relative long-lasting immunity, make them a popular choice of adjuvant. The enhanced immune response they elicit is thought to be as a result of a slow-release (depot) effect based on their ability to adsorb antigens to their surface. Recent evidence also suggests activation of the innate immune response as mechanism of action. Following uptake by antigen-presenting cells, the antigen is released slowly (thus prolonging exposure to the immune system) and it stimulates inflammasomes responsible for activating inflammatory responses. Aluminium hydroxide is also used for its ability to adsorb and inactivated bacterial endotoxin, but it is important to note that this ability is lost as an after effect of freezing.

Emulsions are formed when two liquids are brought together that are unable to form a homogenous mixture. This allows the liquid present in lesser volume to form small droplets within the other and this mixture is then stabilised by adding an interfacial surfactant layer. There are three emulsion adjuvants, the first being *water in oil (W/O)* that contains water droplets in an oil phase. Antigen is contained in these water droplets and the oil acts as a depot, slowly releasing antigen and enhances the immune response by decreasing clearance time and prolonging antigen exposure. Another very popular adjuvant for animal vaccines are *oil in water (O/W)* formulations. In contrast with W/O formulations, they do not increase the immune response by creating a slow-release depot, but rather by increasing inflammatory reaction and stimulating overall immune response. These formulations are believed to be more potent adjuvants for viral vaccines than aluminium salts. Lastly the most advanced emulsion adjuvants are *water in oil in water (W/O/W)* formulations. They contain the benefits of both the aforementioned emulsion adjuvants and have a slow release as well as an immediate stimulatory effect, thus creating a prime-boost effect with a single injection.

Saponins are naturally occurring amphipathic compounds with many pharmaceutical uses, one of which is its use as an adjuvant. Their structure and size promote antigen phagocytosis by antigen-presenting cells and the sugar group in saponins bind to lectins on the antigen-presenting cells that consequently stimulate them to secrete cytokines that promote activation of cell-mediated and humoral immune pathways.

Toll-like receptor (TLR) agonists are transmembrane receptors (expressed in macrophages and dendritic cells) that recognise certain patterns of fungal-, bacterial- and viral pathogenic components, as well as by-products of cell and tissue destruction. When these receptors are activated, they stimulate cellular activation, phagocytic activation and induction of cytokines that are involved in the development of antigen specific immunity. For example, when TLR4 is stimulated by its natural ligand (or adjuvant agonist) it stimulates antigen presenting cells to secrete various interleukins. Some of these cytokines are responsible for the activation of Th1 cells that assist in the development of cell-mediated-, as well as humoral immunity, against intracellular pathogens such as viruses. Examples of TLR agonists from bacterial origin, is Lipopolysaccharide (LPS) from gram negative cell membranes, and the protein flagellin. These agents have not been included in commercial adjuvants to date but are garnering attention from the scientific research community due to their promising potential as adjuvants.

Cytokines are signalling molecules secreted by specific cells of the immune system. They are key components of the immune response stimulated by TLR agonists and the direct addition of these molecules as adjuvants is currently being investigated. Various cytokines could stimulate immunity against either intracellular or extracellular pathogens, depending on their physiological function.

Polymers are (natural or synthetic) compounds of relatively high molecular weight, consisting of large numbers of repeated linked units. Chitosan is an example of a natural polymer utilised in vaccine technology, as well as other pharmaceutical applications. The exact mechanism of action by which polymers act as adjuvants is not yet determined, but one theory suggest that they create a slow-release depot by entrapping antigens in their large cross-linked structure.

Adjuvantation provides many opportunities for future research and development such as inclusion of novel antigens and the combination of adjuvants to stimulate immunity via various pathways. In the years to come, it would be prudent to focus on the development of adjuvants that support single dose vaccination strategies with extended durations of immunity, as well as vaccines that are able to produce immunity in the presence of maternal antibodies.[6]
It is evident that adjuvants will play an increasingly important role as we strive to overcome existing immunologic barriers and find solutions to improve the efficacy, safety and convenience of vaccine technology.

REFERENCES

1. Apostólico JD, Lunardelli VA, Coirada FC, Boscardin SB, Rosa DS. Adjuvants: classification, modus operandi, and licensing. Journal of immunology research. 2016 Jan 1; 2016.
2. World Health Organization. The power of vaccines: still not fully utilized. Available on: https://www. who. int/publications/10-year-review/vaccines/en/[Last accessed: 2019, Apr 23]. 2020.
3. Pasquale AD, Preiss S, Silva FT, Garçon N. Vaccine adjuvants: from 1920 to 2015 and beyond. Vaccines. 2015 Jun;3(2):320-43.
4. Burakova Y, Madera R, McVey S, Schlup JR, Shi J. Adjuvants for animal vaccines. Viral immunology. 2018 Jan 1;31(1):11-22.

5. Kindt TJ, Goldsby RA, Osborne BA. Kuby Immunology Sixth Edition. WH Freeman and Company: 41 Madison Avenue, New York, NY 10010. 2007. p 63-65, 85.
6. Young AJ. Adjuvants: What a Difference 15 Years Makes!. Veterinary Clinics: Food Animal Practice. 2019 Nov 1;35(3):391-403.
7. McKee AS, Marrack P. Old and new adjuvants. Current opinion in immunology. 2017 Aug 1;47:44-51.