

Henna-Maarit Kyröläinen

VACCINE ADJUVANTS

Faculty of Medicine and Health Technology
Bachelor's Thesis
April 2022

ABSTRACT

Henna-Maarit Kyröläinen: Vaccine Adjuvants
Bachelor's thesis
Tampere University
Degree Programme of Biotechnology and Biomedical Engineering
April 2022

Human immune system is a complex system consisting of innate and adaptive immune responses that together provide protection against internal and external dangers. Components of the immune system, including the cells and molecules, have the ability to recognize and tell apart body's own structures from dangerous, foreign structures. Vaccines use this capability to stimulate the innate immune response and antigen presenting cells (APCs) leading to activation of adaptive immune response and protection against a specific antigen. Traditional vaccines are highly immunogenic whole-pathogen vaccines that contain inactivated or weakened pathogens. Vaccine development has progressed in a direction where purified components of pathogens are used in vaccines. This has lowered the immunogenicity of vaccines but at the same time increased their tolerability.

Adjuvants are substances used in vaccines to increase their immunogenicity and to enhance the formation of antigen-specific immune responses. The first adjuvants, aluminium salts, were introduced to vaccines almost a hundred years ago. Adjuvants help the antigen to activate the innate immune response using one or more mechanisms of action. The action mechanisms are mostly local effects induced at the injection site that affect the type, quality and magnitude of the adaptive immune response.

However, adjuvant action mechanisms are not fully understood, but the increased knowledge and research on the immune system and what the adjuvant's role is in the immune response have led to the development of more advanced and complex adjuvant systems and better vaccine design. The discovery of new possible adjuvants could be the key to developing more effective vaccines against more challenging pathogens. This thesis is a literature review that gives an outlook on human immune response, history of vaccination and current vaccine technologies and discusses the importance of adjuvants in vaccines. It also reviews adjuvant action mechanisms and presents few adjuvants used in marketed vaccines.

Keywords: immune system, adaptive immunity, innate immunity, vaccine, antibody, adjuvant

The originality of this thesis has been examined using the Turnitin OriginalityCheck service.

TIIVISTELMÄ

Henna-Maarit Kyröläinen: Rokoteadjuvantit
Kandidaatintutkielma
Tampereen yliopisto
Bioteknologian ja biolääketieteen tekniikan tutkinto-ohjelma
Huhtikuu 2022

Ihmisen immuunijärjestelmä on monimutkainen systeemi, joka muodostuu luontaisesta ja hankitusta immuunivasteesta. Nämä vasteet yhdessä suojaavat kehoa sisäisiltä ja ulkoisilta vaaroilta. Immuunijärjestelmän osilla, kuten soluilla ja molekyyileillä, on kyky tunnistaa ja erottaa kehon omat rakenteet vaarallisista, vieraista rakenteista. Rokotteilla hyödynnetään tätä kykyä stimuloimalla luontaista immuunivastetta ja antigeenia esitteleviä soluja, mikä johtaa hankitun immuunivasteen aktivointiin ja muodostaa suojan tiettyä taudinaiheuttajaa vastaan. Perinteiset rokotteet sisältävät kokonaisia inaktivoituja tai heikennettyjä taudinaiheuttajia, mikä tekee niistä erittäin immunogeenisiä. Rokotekehitys on edistynyt suuntaan, jossa rokotteet sisältävät puhdistettuja taudinaiheuttajien osia. Tämä on johtanut rokotteiden immunogeenisyyden alenemiseen, mutta samanaikaisesti lisännyt niiden siedettävyyttä.

Adjuvantit ovat aineita, joita lisätään rokotteisiin parantamaan niiden immunogeenisyyttä ja vahvistamaan antigeenispesifisen immuunivasteen muodostumista. Ensimmäiset adjuvantit, alumiinisulolat, otettiin käyttöön rokotteissa lähes sata vuotta sitten. Adjuvantit auttavat antigeenia aktivoimaan luontaista immuunivastetta hyödyntämällä yhtä tai useampaa toimintamekanismia. Yleensä adjuvantin toiminta perustuu paikalliseen annostelualueella tapahtuvaan mekanismiin, joka vaikuttaa hankitun immuunivasteen tyyppiin, laatuun ja suuruuteen.

Siitä huolimatta, että moni asia immuunijärjestelmästä ja adjuvantin roolista on säilynyt mysteerinä, lisääntynyt tieto ja tutkimus ovat johtaneet kehittyneempien ja monimutkaisempien adjuvanttijärjestelmien sekä parempien rokotteiden kehitykseen. Uusien mahdollisten adjuvanttien löytäminen saattaa olla avain tehokkaampien rokotteiden kehittämiseen, myös haastavampia taudinaiheuttajia vastaan. Tässä kirjallisuuteen perustuvassa tutkielmassa annetaan yleiskatsaus ihmisen immuunivasteesta, rokotteiden historiasta ja nykyisistä rokoteteknologioista sekä pohditaan adjuvanttien merkitystä rokotteissa. Tutkielmassa käydään läpi myös adjuvanttien toimintamekanismeja ja esitellään muutamia markkinoilla oleviin rokotteisiin lisättyjä adjuvantteja.

Avainsanat: immuunijärjestelmä, hankittu immunitaetti, luontainen immunitaetti, rokote, vasta-aine, adjuvantti

Tämän julkaisun alkuperäisyys on tarkastettu Turnitin OriginalityCheck -ohjelmalla.

PREFACE

This Bachelor's Thesis is made at Tampere University as a part of my Biotechnology and Biomedical Engineering studies.

I want to express my gratitude to my thesis instructor Minna Hankaniemi for giving me this interesting topic as well as for her comments and instructions on how to improve my work. She gave me a direction when I needed one.

I also want to thank my friends and family who supported me during the writing process and helped me clear my thoughts just by listening and being there for me.

Tampere, 26th of April 2022

Henna-Maarit Kyröläinen

CONTENTS

1. INTRODUCTION	6
2. HUMAN IMMUNE SYSTEM AND IMMUNE RESPONSE.....	7
2.1 B cells	8
2.2 T cells	8
2.3 Immune response	9
2.4 Complement system	10
2.5 Pathogen recognition receptors	12
2.5 Cytokines and chemokines	13
3. WHAT IS A VACCINE.....	14
3.1 A brief history of vaccination	14
3.2 Vaccine types	15
3.2.1 Conventional vaccines	15
3.2.2 Next-generation vaccines.....	17
4. WHAT IS KNOWN ABOUT VACCINE MEDIATED PROTECTION MECHANISMS	18
5. VACCINE ADJUVANTS.....	21
5.1 Adjuvant action mechanisms	22
5.2 Adjuvant types	24
6. CONCLUSION	26
7. REFERENCES	28

LIST OF ABBREVIATIONS AND SYMBOLS

APC	antigen presenting cell
ATP	adenosine triphosphate
CTL	cytotoxic T lymphocyte
CLR	C-type lectin receptor
CpG-ODN	CpG oligodeoxynucleotides
CR	complement receptor
CRP	C-reactive protein
DC	dendritic cell
dsRNA	double stranded RNA
DAMP	damage-associated molecular pattern
GPCR	G-protein coupled receptor
IFN- γ	interferon-gamma
Ig	immunoglobulin
IL	interleukin
IL-1Ra	IL-1 receptor antagonist
LPS	lipopolysaccharide
LRR	leucine-rich repeat
MAC	membrane attack complex
ManR	mannose receptor
MBL	mannose binding lectin
MHC	major histocompatibility complex
mRNA	messenger RNA
MyD88	myeloid differentiation factor 88
NK cell	natural killer cell
NLR	nucleotide-binding oligomerization domain (NOD)-like receptor
NLRP3	NLR family pyrin domain-containing 3
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
RIG-I	retinoic acid-inducible gene I
ssRNA	single stranded RNA
TCR	T cell receptor
Th cell	T helper cell
TIR	Toll-interleukin 1 receptor-resistance
Tk cell	T killer cell
TLR	toll-like receptors
VLP	virus-like particle

1. INTRODUCTION

Vaccines have been used to prevent and treat infectious diseases such as viral and bacterial infections for centuries to improve public health and increase the life expectancy. They protect the host by stimulating the immune system by engaging with the innate immune system to activate the adaptive immune response (Keshavarz-Fathi and Rezaei, 2019) in order to create a specific protection against a specific pathogen or disease (Di Pasquale et al., 2015). Efficient and long-lasting protection and immunization achieved through vaccination requires that both the innate and adaptive immune system are activated, and that effector and memory cells are produced to form a memory in case the threat is encountered again.

In addition to infection caused by microbes, studies have shown that the possibilities for vaccines are much greater than originally thought due to the development of technology and science. These possibilities lay in areas including cancer research for therapeutic vaccines but also in other diseases where immune system is involved for example autoimmune diseases or graft rejection (Keshavarz-Fathi and Rezaei, 2019). Furthermore, the advances in vaccine technology have changed the way we produce vaccines. For a while, vaccines were made solely using live-attenuated or whole-pathogen preparations which have high immunogenicity but could still cause a disease after vaccination. The development of recombinant technologies have opened new possibilities where vaccines could contain pathogen subunits, for example toxoids or fragments, or purified antigens, instead of live pathogens, in suspension with preservatives and stabilizers (Di Pasquale et al., 2015).

Although recombinant vaccines might have improved safety and are more tolerable, they also usually have decreased immunogenicity compared to traditional vaccines which means that the protection received by vaccination may be incomplete. To tackle the problem regarding low immunogenicity, adjuvants were discovered to enhance the efficacy of recombinant vaccines. Adjuvants are described as substances added to vaccines to induce a stronger immune response (Wu and Liu, 2021). They are thought to activate the innate immune response through a variety of mechanisms and initiate the downstream adaptive immune activities (Di Pasquale et al., 2015). While much knowledge has been gained regarding how the immune response works and how the adjuvants affect it, there are several mysteries and knowledge gaps that require solving.

The purpose of this thesis is to review the basics of how human immune system works, what vaccines are and how they help to generate an immune response to protect the host, as well as what are adjuvants and what is their mechanism of action in vaccines and in immune response, the focus being on microbial infections.

2. HUMAN IMMUNE SYSTEM AND IMMUNE RESPONSE

Human immune system has developed to become a dynamic defense mechanism whose cells and molecules can separate body's own and foreign structures as well as safe and harmful substances (Hedman et al., 2011). The structures of immune system act in a specific order and highly organized to elicit appropriate and well-organized reactions that can be switched off if necessary. Both overactivity and deficient action can have severe impact on individual's health. The immune response can be roughly divided into two main parts: the innate and adaptive immune responses (Hedman et al., 2011).

The innate immune response, also known as natural immunity, is the early stage defense mechanism that consists of physical and chemical barriers such as epithelia and chemicals produced at epithelial surfaces, low pH, leucocytes such as phagocytic cells, dendritic cells (DCs) or lymphocytes, microbial peptides, complement system as well as many other structures and molecules (Hedman et al., 2011). The reactions of innate immunity are typically fast and recur quickly but similarly each time for the innate immunity does not form a memory like adaptive immunity. In addition, the molecules taking part in the reactions have only a limited ability to recognize target structures and so the general response is based on common structures of group of related microbes (Hedman et al., 2011). These structures can for example be parts of the virus protein capsid, bacterial cell wall or polysaccharides. The cells of innate immune system act first by recognizing a microbe and then progressing to phagocyte, kill and process it. After this the cells will migrate via lymph nodes to present these processed structures, antigens, to adaptive immune cells. The cells working in innate immunity are granulocytes such as neutrophils, eosinophils, basophils and mast cells, dendritic cells, natural killer cells and monocytes that can develop into macrophages.

On the other hand, adaptive immune response, also known as acquired immunity, is the later, more specific defense mechanism that can respond to repeated exposures to the same matter and tell the difference between different pathogens. The adaptive immunity relies on cells called lymphocytes and antibodies that recognize antigens. Adaptive immune response can further be divided into cellular and humoral immunity mediated by T cells and antibody-producing B cells, respectively (Pollard and Bijker, 2021). Adaptive immunity produces a memory which means that if the same pathogen is encountered again, the memory cells will be activated, and the pathogen will be destroyed effectively. Adaptive immunity is activated by the cells of innate immune response and in turn, it can also activate the cells of innate immune response so that they can become more efficient in their actions against pathogens.

2.1 B cells

B cells are lymphocytes that drive the humoral immunity of the adaptive immune response. B cells develop in the bone marrow (Walsh and Bolland, 2014) and they circulate in lymph nodes and spleen while they are not activated (Cruse et al., 2004). They can interact with T cells and be activated by them at extrafollicular sites where T cells and DCs are present, or they can be activated without antigen presentation by T cells (Cruse et al., 2004). When B cell develops from a stem cell, it is released from the bone marrow as an immunoglobulin M (IgM) positive B cell that migrates into lymph nodes. When B cell faces an antigen that can bind to its immunoglobulin receptor, it is activated and can mature and differentiate into plasma cells that produce antibodies against pathogens, and memory cells that persist a long time in the body and maintain a memory of the first encounter with the pathogen for the specific antigen and they will produce antibodies when the pathogen is encountered again. B cell activation induces a class change to another immunoglobulin, IgG.

Antibodies (i.e., immunoglobulins) consist of light and heavy chains that form an Y shaped molecule. There are five antibody categories IgM, IgG, IgA, IgD and IgE. Antibody molecules have a constant region and variable region. The variable region is responsible for mediating the antigen recognition while the constant region stimulates the innate and adaptive immune response (Hedman et al., 2011).

2.2 T cells

T cells are lymphocytes that develop in bone marrow but migrate to thymus for maturation and mediate the cellular immunity of adaptive immune response. They help and guide the activity of other cells of immune response. T cells can be categorized as either cytotoxic killer T cells (T_k cells or CTL) or helper T cells (T_h cells) depending on their surface structure CD8⁺ or CD4⁺, respectively (Varkila and Hurme, 1992). Both structures are important for antigen recognition because the T cell receptor (TCR) on the T cell surface recognizes the antigen on the antigen presenting cell (APC), such as macrophage or dendritic cell, through the major histocompatibility complex (MHC) Class I molecules with CD8⁺ cells and MHC Class II with CD4⁺ cells (Varkila and Hurme, 1992).

Th cells have subtypes which can be differentiated from each other by their cytokine production profiles in addition to their key purpose. The best described subtypes, T helper 1 (Th1) and T helper 2 (Th2) cells, are important factors in inducing cellular and humoral immunity. The most important difference between the two subtypes is that Th1 cells produce cytokines interleukin 2 (IL-2) and interferon-gamma (IFN- γ) while Th2 cells produce interleukins 4, 5, 6, and 10 but not the other way around (Varkila and Hurme, 1992). This difference comes to play in immune defense for in most cases, these cells and their cytokines have opposite effects and even inhibit each other. Another

difference is that Th1 cells have been found to be important especially for cellular immunity while Th2 cells are connected to B cell activity and further to the regulation of antibody production. The inflammatory mediators produced can for example stimulate other lymphocyte activity, affect the antibodies produced and inhibit the immune response in some form. Th1 and Th2 cell activity has been shown to differ in different infectious diseases and that depending on the cell profile, the disease can be very different. Other subtypes in different tissue surfaces are T helper 17 (Th17) cells which are found from mucosal surfaces, for example gut and lung, and T follicular helper cells in secondary lymphoid organs (Pollard and Bijker, 2021).

2.3 Immune response

When the body is exposed to a foreign matter, the cells of the innate immune system, for example neutrophils, dendritic cells, monocytes and macrophages, (Di Pasquale et al., 2015) are able to recognize conserved surface structures such as pathogen-associated molecular patterns (PAMPs) and/or damage-associated molecular patterns (DAMPs) through PRRs such as toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (Pulendran et al., 2021) and C-type lectin receptors (CLRs) (Keshavarz-Fathi and Rezaei, 2019). PAMPs are molecular structures derived from viruses, fungi, bacteria or protozoa and they can for example be polysaccharides, lipopolysaccharides, RNA or DNA (Davies, 2010). DAMPs, on the other hand, are signals released from the host during cellular damage or death (O'Hagan et al., 2020) or tissue damage for example uric acid, nucleotides, adenosine triphosphate (ATP), and cytokines (Awate et al., 2013).

Recognition of foreign matter leads to events of innate immune response that include phagocytosis, killing of infected cells, release of inflammatory mediators, activation of complement system as well as recruitment and activation of lymphocytes. Granulocytes arrive first to the infection site followed by monocytes and lymphocytes. Neutrophils, tissue macrophages, blood monocytes, and dendritic cells can phagocytose the matter, such as pathogens or damaged cells, and enzymatically degrade it in their phagosomes and process it. In addition to phagocytosis, the recognition induces intracellular pathway that leads to production of inflammatory mediators such as cytokines or chemokines which can attract other cells to the site and stimulate their activity as well as initiate the inflammatory response. Especially dendritic cells, but also macrophages and other cells, can present the antigen on their surface via MHC molecules and bring them to lymphocytes in the draining lymph node and activate them. This is one link between the innate and adaptive response.

Natural killer cells (NK cells) can, at the same time, directly kill virus-infected cells or tumour cells, parasites, and bacteria. They can be activated by cytokines produced by macrophages or by interferons. They have intracellular granules that contain cytotoxic enzymes used to destroy cells.

They have both activating and inhibiting receptors on their cell surface which they use, along with MHC molecules, to detect if the cell is normal or not. Cells with abnormal or absent signal from MHC Class I molecule on the target cell surface activates the NK cell to release its granule contents into the target cell, directing it towards apoptosis.

Along with the activation of the complement system, these functions form a local inflammation to the infected area. APCs migrate to the draining lymph node to present the antigens via MHC molecules to recruit and activate lymphocytes (Hedman et al., 2011). Activated CD8⁺ T cells are able to kill cells by either releasing cytokines that have anti-microbial and anti-tumour properties, releasing cytotoxic granules which contain enzymes that can damage the target cell wall or proteins inside the cell, or kill cells directly via Fas ligand and its receptor (<https://www.immunology.org/public-information/bitesized-immunology/cells/cd8-t-cells>, 8.2.2022). The T cell expresses the ligand on its surface and when it binds to the target cells receptor, an intracellular caspase pathway is activated which leads to apoptosis of the target cell. They also secrete IFN- γ that can stimulate macrophages. CD8⁺ cells also along with the B cells affect the immunological memory by forming memory and effector cells. CD8⁺ memory cells can rapidly proliferate when they come across a pathogen (Pollard and Bijker, 2021) and CD8⁺ effector cells can eliminate infected cells efficiently. CD4⁺ T cells, on the other hand, once activated secrete specific cytokines depending on the subtype that help activate B cells and other cells and direct their function by stimulation or inhibition. Activated B cells mature and develop into plasma cells that produce antibodies and to memory cells that maintain the antigen specificity for longer period. The produced antibodies can directly kill the microbes by using the complement system to opsonize them. This acts as a marker for phagocytic cells and NK cells to destroy them but also, causes the cells to release inflammatory mediators that induce inflammatory reaction. The antigen can also make its way inside the B cell which is presented on the MHC molecule on B cell surface. This further stimulates the T cell and causes it to produce cytokines and co-stimulate the B cell which leads to efficient activation of the B cell (Hedman et al., 2011).

2.4 Complement system

In addition to PRRs, a system called complement is an important link between innate and adaptive immunity that leads to recognition and termination of microbes in body fluids (Bennett et al., 2017). The complement system is part of the innate immune system and comprises of plasma proteins that together attack against extracellular forms of pathogens. Most of the proteins involved in the complement cascade are produced in liver or macrophages and they include both effector and inhibitory molecules. The complement system is activated either spontaneously by specific pathogens or by antibody binding to the pathogen (Janeway et al., 2001). This leads to the coating of the pathogen surface by complement proteins, a process known as opsonization, which assist the removal of pathogens by phagocytosis or by killing the pathogen directly. The complement can affect

the humoral immunity by lowering the threshold for B cell activation and that way increase the production of antibodies but at the same time also increase antigen presentation and even affect the cellular immunity (Bennett et al., 2017).

The complement system can be described as a “chain reaction” where the product of a previous reaction is the effector of the next one leading to a formation of a cascade. Complement system can be activated via three pathways: alternative, classical or lectin pathway (Bennett et al., 2017). All three pathways are activated by different mechanisms although, it usually occurs either via proteolytic cleavage or binding to a previous effector. They also have a common feature - each pathway leads to a cleavage of C3 protein in the complement cascade (Bennett et al., 2017) and to the formation of C3 and C5 convertases. After these the outcome of the cascade is independent of the pathway activated. In other words, each cascade can lead to opsonization, activation of membrane attack complex (MAC), and recruitment and activation of lymphocytes leading to local inflammation.

The alternative pathway is activated spontaneously when C3 is hydrolysed. The C3 is cleaved to form C3b and C3a from which C3b is deposited onto the microbe and leads to opsonization of the microbe cell surface (Bennett et al., 2017). This can lead to the production of C5 convertase enzymes that can form C5a and C5b by C5 cleavage which can induce the formation of MAC to mediate cell lysis via pore formation. At the same time, C5a and C3a can recruit and activate leukocytes and lead to the destruction of microbes by leukocytes. They can also help regulate T cell immunity. The classical pathway is activated when antibodies IgG or IgM are bound to the microbial antigens (Bennett et al., 2017). It is also possible that the pathway is activated via C-reactive protein (CRP), immune complexes, or cell-free chromatin. This eventually leads to the deposition of C3b on to the microbe’s surface which then acts as a signal that can be recognized by phagocyte C3b receptor leading to recruitment of cells and phagocytosis of the microbe. The lectin pathway, as the name suggest, is activated by mannose binding lectin (MBL) that binds to specific carbohydrate patterns on to microbe’s cell surface (Bennett et al., 2017). This pathway also leads to the C3b opsonization, formation of MAC and phagocytosis of the microbe.

The C3b can be recognized by a complement receptor 1 (CR1). Complement receptors are G-protein coupled receptors (GPCRs) which are located at the cell surface of several immune cells (Bennett et al., 2017). Complement receptors such as CR1, CR2, CR3 and CR4 can interact with each other and take part in functions such as antigen presentation, stimulation of lymphocytes and phagocytosis. The complement system also needs regulation to control its activation which is achieved by inhibitory proteins on the host cell surface that act as signals to prevent the pathway activations. There are also other inhibitory proteins that can bind some of the cascade mediators to

help convert the key actors into inactive forms. These actions slow the activation and the amplification of the complement system.

2.5 Pathogen recognition receptors

PRRs are an important part in activating the immune response. They can be found from cells such as phagocytes and APCs either from the cell surface or from intracellular membrane structures as well as from cytosol. There are also some PRRs that are secreted receptors found from blood and lymph (Davies, 2010). PRRs recognise different conserved pathogen structures (PAMPs) (Hedman et al., 2011) but also structures associated to and released in cellular damage (DAMPs). Additionally, they can also recognise adjuvants as danger signals. By recognizing these structures, the receptors activate the cells by inducing intracellular signalling leading to inflammation mediator (e.g., interferon and cytokine) production and activation of innate immune system to fight off the pathogen.

TLRs are membrane bound receptors essential in initiating innate immune response. They have an extracellular leucine-rich repeat (LRR) domain that recognises PAMPs, a transmembrane domain and a cytoplasmic Toll-interleukin 1 receptor-resistance (TIR) domain that directs the intracellular signalling through adaptor molecules such as myeloid differentiation factor 88 (MyD88) (Davies, 2010). They can be expressed in several different cell types but mostly in APCs like macrophages and dendritic cells. Ten different TLRs are known in humans (Hedman et al., 2011). TLRs 1, 2, 4, 5, 6 and 10 are expressed on the cell surface while TLRs 3, 7, 8 and 9 are expressed on endosomes (O'Hagan and De Gregorio, 2009; Pulendran et al., 2021). Some TLRs can recognize bacteria or protozoans while some recognize fungi or viruses. In the case of viruses, for instance, TLR3 can detect double stranded RNA (dsRNA) while TLR7 and TLR8 detect single stranded RNA (ssRNA) (Hedman et al., 2011). Different TLRs can respond to very different PAMPs, and their activation always leads to slightly different inflammatory and cytokine response in the cells which, in the end, causes the antimicrobial response and cytokine directed immune response to be different. TLRs have been considered as good candidates for vaccine adjuvants because they can link the innate and adaptive response together.

On the other hand, RIG-I and NLR are cytosolic PRRs (Davies, 2010). RIG-Is are expressed in almost all human cells, and they can detect both viral ssRNA and dsRNA structures. NLRs can, for example, detect peptidoglycan from gram positive bacteria but NLR family also includes a member, NLR family pyrin domain-containing 3 (NLRP3), that activates an inflammasome in cells due to metabolic stress leading to IL-1 β and IL-18 production as well as apoptosis (Hedman et al., 2011).

In addition to these PRRs, there are also secreted PRRs such as CLR for example mannose receptor (ManR). They recognize polysaccharide structures, such as mannose or N-

acetylglucosamine, from the pathogen surface (Davies, 2010). Dectine-1 and dectine-2 are lectin receptor family members that recognize surface structures from fungi and yeasts (Hedman et al., 2011). Another family member, DC-SIGN, is expressed especially in DCs and it recognized viral glycoproteins. Usually, lectin receptor activation leads to induction of inflammatory response.

2.5 Cytokines and chemokines

Cytokines are mediator molecules that are produced for example during infection from infected cells or due to microbial induced cell stimulation (Hedman et al., 2011). They mediate the communication between the innate and adaptive immunity as well as the cellular and humoral aspects of immune response. There are several different kinds of cytokines that can structurally be peptides, proteins, or glycoproteins. They can either act as inhibitors or activators of the immune response or as growth factors and their effects can range from local to systemic. Cytokines' functions include regulation of homeostasis, activation of antimicrobial defence, regulation, and support of immune response as well as regulation of cell growth and differentiation. Some cytokines have been studied as potential vaccine adjuvants.

Chemokines are chemotactic cytokines that can attract cells to their location. For example, during infection or inflammation reaction they can selectively attract leucocytes to peripheral tissues. Their selectivity is based on different chemokine receptors that leucocytes express on their cell surface and each leucocyte has their own specific collection on receptors. Binding to a chemokine activates the cell and changes its morphology allowing it to penetrate the blood vessel wall between endothelial cells to access the peripheral tissues.

IFNs are cytokines that communicate viral infection to cells and enhance the actions to remove the virus from the body. IFNs can be categorized into three groups: type I IFNs (IFN- α , IFN- β and IFN- ω), type II IFNs (IFN- γ) and type III IFNs (IFN- λ 1-3) (Hedman et al., 2011). Type I IFNs stimulate the expression of several antiviral molecules leading to decreased viral proliferation. Additionally, they inhibit cell growth and division but can increase Th1 cytokine receptor expression. Type II IFNs increase MHC molecule expression and activation of macrophages which enhances the cells' ability to present antigens. Type III IFNs have some antiviral and cell activating effects.

Proinflammatory cytokines, such as IL-1, IL-6, TNF- α and Th1 cytokines (e.g., IL-12, IL-18 and IFN- γ), are cytokines that activate or enhance an inflammatory reaction (Hedman et al., 2011). The first three activate target cells leading to further chemokine and proinflammatory cytokine production. In addition, the cells capacity to react to extracellular stimuli is enhanced along with the expression of surface antigens and adhesion molecules. Th1 cytokines enhance the inflammatory response and

activate target cells to produce more chemokines, inflammatory cytokines and can affect, for example, T cell activation, polarization, and memory.

On the other hand, anti-inflammatory cytokines, such as IL-1Ra (IL-1 receptor antagonist), TGF- β and Th2 cytokines (e.g., IL-10 and IL-4), are cytokines that inhibit or weaken the inflammatory reaction (Hedman et al., 2011). They prevent an excessive cell stimulation and tissue damage caused by inflammation as well as reduce the production of inflammatory cytokines in target cells. Especially Th2 cytokines regulate the development of humoral response of adaptive immunity. For example, IL-4 functions as a growth and differentiation factor to both Th2 and B cells.

3. WHAT IS A VACCINE

Vaccination is a form of active immunization which means that in an unimmunized person an antibody production is induced against a particular antigen. A vaccine can be considered as a biological product used to safely stimulate immune response to acquire protection against a pathogen induced infection or disease following a later exposure to the pathogen (Pollard and Bijker, 2021). The immune response is activated by antigens included in the vaccine. They are components of the pathogen, for example carbohydrates or proteins, that can be either synthetically produced or obtained straight from the pathogen. There are also other components included in the vaccine to act as preservatives, emulsifiers or stabilizers (Pollard and Bijker, 2021).

Vaccines act by inducing antibodies against the antigen (Davies, 2010). By triggering the immune response, vaccines activate the innate immune response and APCs leading to adaptive immune response (Keshavarz-Fathi and Rezaei, 2019). Vaccination produces an immunological memory that protects the host when the same pathogen is encountered again by inducing a faster immune reaction to prevent the pathogen from causing harm to the host. Furthermore, adjuvants are added to vaccines to ensure the activation of immune response by improving the immunogenicity of a vaccine.

3.1 A brief history of vaccination

Protection against infectious diseases and the history of vaccination dates back to over 2000 years. One of the first mechanisms used was inoculation where material from infected person, such as dried pus or scabs, were introduced to the skin or nasal cavity of a healthy person (Di Pasquale et al., 2015). The use of inoculation was based on findings that once a person has recovered from the disease they will not be infected again. This method has been documented to originate either from

India or China before 200 BCE (<https://amhistory.si.edu/polio/virusvaccine/history.htm>, 22.2.2022). Although, inoculation might have protected some individuals, it most often led to severe diseases and high death rates. Smallpox was the first disease to be eradicated by using the principle of vaccination, the more safe and effective form of active immunization than inoculation.

The context of immunization and the idea of vaccination was developed by Edward Jenner in late 1790s. Using inoculation in demonstrations, he observed that person who suffered from mild cowpox infection did not develop smallpox and for this reason the material from cowpox could be used for immunization against smallpox (Pollard and Bijker, 2021). A century later, the studies of Louis Pasteur over rabies vaccine created a positive force that led to a rapid development of new vaccines against diseases such as diphtheria and pertussis (Pollard and Bijker, 2021). These vaccines developed in the early 1900s consisted of inactivated pathogens or bacterial toxins. The production of toxoid vaccines was based on findings that toxins maintain their high immunogenicity even when modified into a non-toxic form (Di Pasquale et al., 2015). Other advances met in this century were the use of cell cultures for pathogens in the vaccine development and introduction of national vaccine programmes as a coordinated tool to improve public health. These vaccination programmes are the foundations of the programmes we have today. And partially due to these programmes, has vaccination reduced morbidity and mortality in many diseases to date and will continue to do so in the future.

3.2 Vaccine types

There are many vaccine types on the market today and they can be categorized in different ways. For example, they can be divided by the technology used in their making to form two categories: conventional vaccines and next-generation vaccines (Ghattas et al., 2021). Conventional vaccines include whole-pathogen vaccines and conjugate or subunit vaccines. These all contain one or more antigens taken from weakened or inactivated microorganisms, or components of the pathogen. Next-generation vaccines include virus-like particles, viral vector-based vaccines and nucleic acid vaccines.

3.2.1 Conventional vaccines

Whole-pathogen vaccines are traditional vaccines that were first developed. They contain the entire pathogen that has been either inactivated or weakened. In live-attenuated vaccines, the carefully selected pathogen has been weakened under laboratory conditions so that the virulence is reduced. The pathogen is still “alive” so that it can still infect host cells and replicate in them to cause weak infection that gives an effective protection, however, there is a possibility of mild disease development. Usually these vaccines do not require an adjuvant and can give a lifelong immunity by

one or two vaccine doses (Ghattas et al., 2021; <https://www.niaid.nih.gov/research/vaccine-types>, 2.3.2022). Examples of live-attenuated vaccines are vaccines against measles, rubella, influenza and varicella zoster (Pollard and Bijker, 2021).

Killed whole organism vaccines, also known as whole inactivated vaccines, are produced by killing the pathogen by chemical and/or physical methods using, for example, formaldehyde or hydrogen peroxide as chemical methods and heat or radiation as physical methods (Ghattas et al., 2021). The production of these vaccines requires that, in the case of a virus, the organism can be grown in laboratory conditions and that the inactivation can be proven to be successful. These vaccines are safer than live-attenuated ones because the pathogen is prevented from replicating, but they can generate a broad immune response due to the entire pathogen included in the product. Though, they have a lower immunogenicity which means that several booster shots are required for lasting protection, however, the immunogenicity can be enhanced by the use of adjuvants (Ghattas et al., 2021). Examples of killed-whole organism vaccines are vaccines against poliovirus, hepatitis A virus and Japanese encephalitis virus (Ghattas et al., 2021).

Subunit vaccines include peptide vaccines, toxoid vaccines, polysaccharide, and polysaccharide conjugate vaccines. Peptide vaccines are synthetic products manufactured *in vivo* using different techniques to create around 30 amino acid long peptide. Because of their small molecular size, they require a carefully selected adjuvant as a carrier to enhance their efficiency and immunogenicity. These vaccines are safer than inactivated and live-attenuated vaccines, and the ability to control and tailor the peptide as well as the synthesizing process gives an advantage in the aspects of safety, stability, and efficacy (Ghattas et al., 2021). An example of peptide vaccine is Meningococcal Group B vaccine (Ghattas et al., 2021).

Toxoid vaccines are produced by inactivating toxins secreted by bacteria using chemical or physical methods to alter specific amino acids and cause small conformation changes to the structure of the toxoid to eliminate the harmful effect while still maintaining their immunogenicity, structure and physicochemical properties (Ghattas et al., 2021). Chemical methods like formaldehyde usually provide better outcomes than physical methods when the structural and stability aspects are considered. In toxoid vaccines, the immune response is induced against the disease-causing agent instead of the pathogen differentiating it from other vaccine products. Vaccines against diphtheria and tetanus are examples of toxoid vaccines (Pollard and Bijker, 2021).

Polysaccharide vaccines are based on carbohydrate polymers coating the bacterial cells such as peptidoglycans, glycoproteins, or teichoic acids (Ghattas et al., 2021). These vaccines do not contain living pathogens and they induce a T cell independent immune response because polysaccharides are not presented on MHC molecules (Ghattas et al., 2021). Examples of polysaccharide vaccines

are meningococcal and typhoid Vi vaccines. Although these vaccines have proven to be efficient in adults, they fail to do so in individuals, for example, infants who are more susceptible to bacterial infection (Ghattas et al., 2021). This has been solved by adding adjuvants and conjugating the polysaccharide into a carrier protein (e.g., tetanus toxoid) by covalent bonds to increase the efficacy of the vaccine. The difference of the action mechanism of polysaccharide and these polysaccharide conjugate vaccines is that in the latter both the protein and the polysaccharide are presented on MHC molecule leading to Th cell activation and in the end a stronger B cell memory. Examples of these conjugate vaccines are haemophilus B, meningococcal and pneumococcal vaccines (Ghattas et al., 2021).

3.2.2 Next-generation vaccines

Virus-like particles (VLPs) are particles that have been made to imitate a virus in their shape, size and surface epitopes (Ghattas et al., 2021), but do not contain viral genome which means that they are not able to replicate. However, they induce an efficient immune response because VLP resembles a real virus. They are made using recombinant protein technology where the recombinant protein product is produced in bioreactors, for example in yeast, plant, bacterial or mammalian cells where the capsid proteins arrange spontaneously into virus-like particles. VLPs are safe to produce and handle which differs from inactivated and live-attenuated vaccines. In addition, the production is easily reproducible, and this technique allows the vaccine to be tailored throughout the manufacturing process to achieve an optimal immune response. However, there may be challenges regarding the purification, storage, and design processes. Adjuvants can be added to these vaccines to strengthen the immune response. Vaccines using VLPs include human papillomavirus vaccine and it is being studied against Chikungunya and Zika Virus (Ghattas et al., 2021).

Viral vector vaccines use a genetically modified virus, such as adenovirus, to transport target antigen coding DNA into the host cells. The DNA has been included into the viral genome and the virus vector act as a carrier that efficiently invades host cells and causes a short-term viral protein production which induces immune response against the antigen. The viral vectors can either be able to replicate or not. The former causes an infection, but the latter does not and is therefore considered to be more safe and easier to manufacture. Viral vector vaccines used today are fore example Ebola Zaire vaccine and SARS-CoV-2 vaccine (Ghattas et al., 2021).

DNA vaccines are based on the concept of transferring the antigen coding DNA into the host target cells. They are also large, polyanionic and sensitive to nucleases and they can induce both the cellular and humoral arms of the immune response (Ghattas et al., 2021). Thought, it possesses challenges regarding how the DNA is transferred into the nucleus effectively enough and how we can ensure sufficient long-term gene expression as well as a safety concern of the possibility of the

DNA integrating into the genomic DNA and to get a proper T and B cell response, booster shots are required. These struggles are reflected in practice for there has been multiple experimental DNA vaccines for humans being studied against bacteria, parasites, viruses, and cancer, but only DNA vaccine based on DNA plasmid against SARS-CoV-2 (ZyCoV-D) has been authorized for emergency use.

Messenger RNA (mRNA) vaccines are based on a concept of transporting mRNA molecule into host target cell cytoplasm that codes a specific antigen. These mRNA molecules are genetically modified, for example switching uridine to N1-methyl-Pseudouridine, so that it remains functional for a longer period. The transportation of mRNA inside the cell is enough to begin the protein production and being highly immunogenic, they promptly induce an immune response through local chemokine production. In addition, the transportation into cells can be enhanced by using lipid nanoparticles as was done in the case of SARS-CoV-2 vaccines, the first mRNA vaccines approved for clinical use (Ghattas et al., 2021). mRNA vaccines can be considered to be easier and less costly to produce than DNA vaccines but as a down side, they have limitations regarding the stability at room temperature, requirement of cold chain transport and high reactogenicity (Ghattas et al., 2021).

4. WHAT IS KNOWN ABOUT VACCINE MEDIATED PROTECTION MECHANISMS

Many vaccines that are on the market, mediate protection through the induction of antibodies (Pollard and Bijker, 2021), but to receive a proper immunization, both the humoral and cellular aspects of the adaptive immune response need to be stimulated (Keshavarz-Fathi and Rezaei, 2019). This is achieved by activating both the innate and adaptive immune systems collectively and producing effector and memory cells. Although antibody production is the main part of vaccine induced immunization, most vaccines can also induce the cellular, T cell response (Pollard and Bijker, 2021). Antibody producing B cells prevent an infection and they are activated by the help of CD4+ T cells whereas CD8+ T cells control and remove an established infection (Pollard and Bijker, 2021). In Figure 1, there is a short summary of vaccine induced immune response.

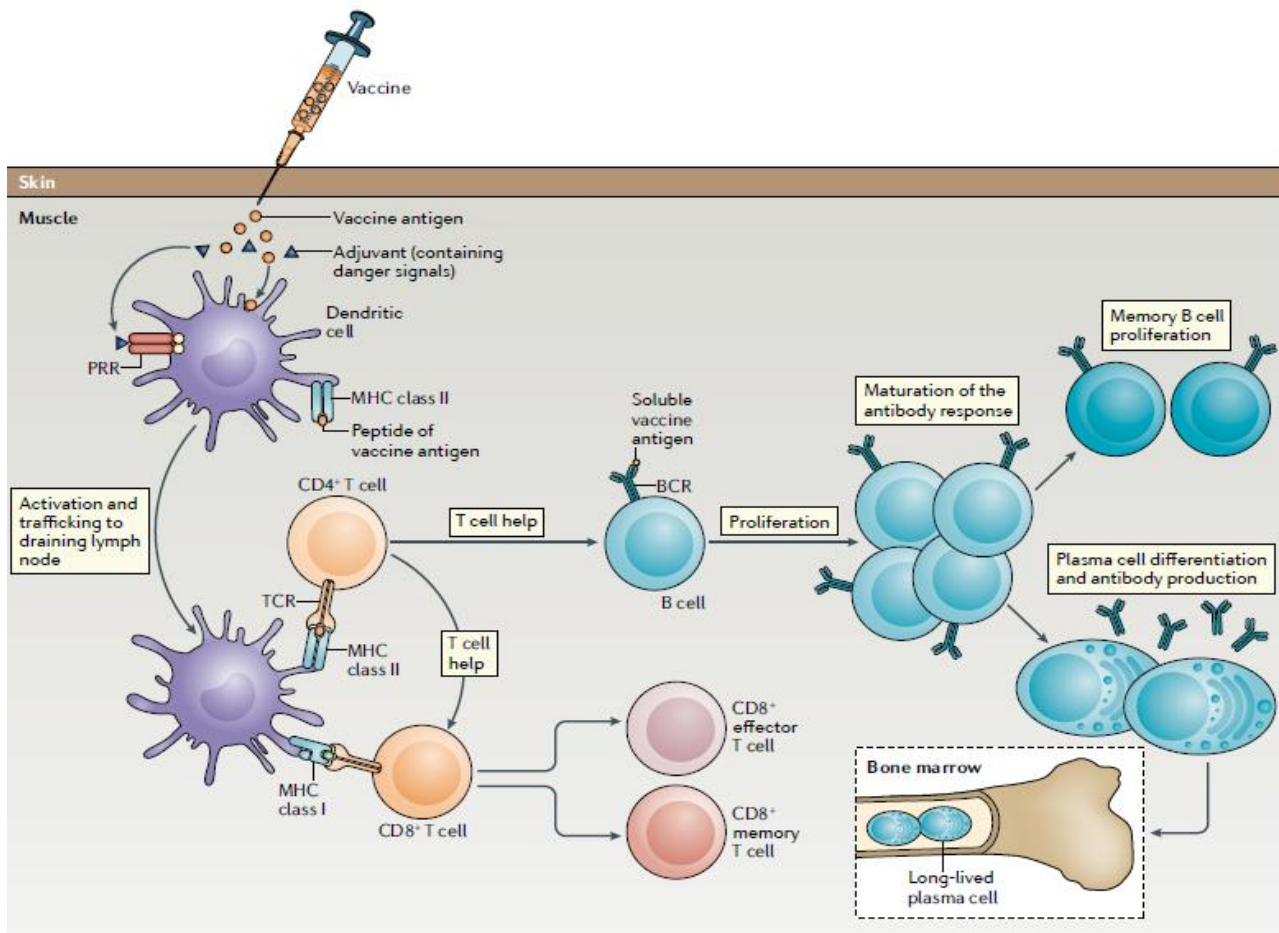


Figure 1. Formation of an immune response to intramuscular vaccine administration. The vaccine antigen is taken up by dendritic cells (DCs) which are activated through PRRs by adjuvants. DCs migrate into draining lymph nodes where they present the antigen on MHC molecule and activate the T cells through TCRs. B cells are activated with the help of T cells and costimulatory signal. B cell development leads to maturation of the antibody response and production of different antibody isotypes. Short-lived plasma cells produce specific antibodies for the antigen which causes a quick rise in serum antibody levels. Memory B cells mediate the immune memory. Long-lived plasma cells in bone marrow niches produce low levels of antibodies for a long period of time. CD8⁺ memory T cells can proliferate rapidly when pathogen is encountered again and CD8⁺ effector T cells eliminate infected cells. PRR, pathogen recognition receptor; MHC, major histocompatibility complex; TCR, T cell receptor; BCR, B cell receptor. (Pollard and Bijker, 2021)

Antibodies can protect the host against free pathogens and infected cells by directly recognizing and/or killing them, through opsonization, neutralization or NK cells, or by inducing inflammation (Burton, 2002; Hedman et al., 2011). Antibodies can bind to infected cells or microbes and mark them which leads to the activation of the classical pathway of the complement system which in turn can lead to the formation of MAC and cell lysis. Opsonization with the antibodies together with

complement proteins attracts phagocytosing cells to destroy the target. Antibodies can also neutralize their target by binding to them which can cause conformational changes to the surface of the target (Burton, 2002) which prevents it from attaching to host cells and gaining entry to the cells. In addition, infected cells and transformed cells can be destroyed by covering them by antibodies to which FcγIII-receptors of an NK cell can bind and activate the NK cell to kill the target in a process called antibody-dependent cellular cytotoxicity. Furthermore, the antibodies can induce inflammation when an IgE molecule on mast cell surface recognizes its antigen causing the mast cell to release inflammation mediators such as cytokines which activate other immune cells leading to pathogen removal.

These antibodies can be categorized to neutralizing and non-neutralizing antibodies. The first act mainly by neutralizing free pathogens and directing them to complement-mediated lysis and phagocytosis (Burton, 2002). They can also have an affect against infected cells because they recognize and bind to envelope molecules that can be found from the pathogen as well as from the infected cell surface. The second usually bind and recognize more the infected cells instead of free pathogens because they can sense molecules on the surface of the cell that are not present in the free pathogen.

However, antibodies are not alone responsible for the protection against a pathogen. In a vaccinated person, the protection originates from CD8⁺ T cells, plasma cells and memory B cells. Along with these cells, also CD4⁺ T cells are needed to activate both CD8⁺ T cells and B cells. CD8⁺ T cells help control and limit viral replication by inducing apoptosis in infected cells (Burton, 2002). These T cells can be induced by the vaccine to be for example virus-specific or they can be induced de novo (Burton, 2002). Long lived plasma cells are responsible for the continuous low secretion of antibodies and maintain the minimum concentration of antibodies in the serum (Hedman et al., 2011). On the other hand, memory B cells are efficiently activated when they encounter the antigen again and can produce quickly large quantities of antibodies even to a small antigen concentration.

Importantly, the main aspect of vaccine induced protection is the production of immune memory. Generating memory cells against a pathogen causes a rapid activation of immune response and increased antibody production against the pathogen when it is encountered again. Depending on the concentration of antibodies produced by memory cells, the incubation time of the infection and the quality of the memory response, the immune memory can either protect against the pathogen or not (Pollard and Bijker, 2021). Against pathogens that have a long incubation period, the immune memory is a good protective system because an immune response can readily develop against the pathogen (Pollard and Bijker, 2021). However, when the infection sets soon after the pathogen invasion, memory response might be deficient and achieving continuous immunity against the pathogen could be hard.

Additionally, vaccines can mediate indirect protection through herd immunity (Pollard and Bijker, 2021). Some people might not be able to take the vaccine and some that are vaccinated do not develop immunity against the pathogen. When enough people are vaccinated in some population, the pathogen transmission can be interfered preventing the infection from spreading in the population and protecting indirectly people who would otherwise be prone to the disease and infection. The degree of vaccination in the population is dependent on the specific pathogen and can have even large differences between different pathogens as well as between different strains of the same pathogen. However, herd immunity could also negatively affect the natural enhancement of vaccinated people and could cause decreasing immunity which would require the use of booster shots (Pollard and Bijker, 2021).

5. VACCINE ADJUVANTS

Adjuvants are substances added to vaccines to increase their immunogenicity (Barclay and Petrovsky, 2017) by activating APCs to achieve a stronger antigen-specific immune response (Keshavarz-Fathi and Rezaei, 2019). In other words, by using adjuvants, a more efficient and long-lasting immune response is achieved. Not long ago, adjuvants were described to be either delivery systems that deliver antigens to immune cells or immune potentiators that affect immune cells and activate them (O'Hagan and De Gregorio, 2009). But being one of the above mentioned does not disclose the other and it has been stated that delivery systems also act as immune response enhancing substances. Furthermore, as more knowledge is gained regarding adjuvants, their mechanism of action and immune response, more specific adjuvants are made and known adjuvants are combined to achieve new effects.

Additionally, there are several reasons why adjuvants are useful in vaccines. Due to the development of new vaccine technologies, the immunogenicity has decreased because of highly purified antigens used in the vaccines (Garçon et al., 2007). Adjuvants can restore and enhance the immunogenicity of the antigen and lead to better results. They also lower the amount of the antigen needed in the vaccines as well as the frequent dosing of the vaccine by improving memory B and T cell responses (O'Hagan and De Gregorio, 2009). Moreover, they can increase antibody titers and effector T cell response as well as help activate the immune response more promptly (O'Hagan and De Gregorio, 2009). Also, they can help broaden the response to tackle the diversity of pathogens to recognize different strains of the same pathogen (Pollard and Bijker, 2021) together with the possibility to produce more complex combination vaccines and vaccines that are more efficient in populations of

immunocompromised or who otherwise have a limited immune response (e.g., elderly and people with chronic illnesses).

Ideal adjuvants are substances that are produced of cheap and easily obtainable components that do not cause any adverse effects and are biodegradable (O'Hagan and De Gregorio, 2009). They should produce the wanted immune response when administered together with the antigen without inducing an immune response against itself. Tolerability is also a key concept in determining the successfulness of the adjuvant alongside safety that is associated to long-term side-effects.

5.1 Adjuvant action mechanisms

Adjuvants can be thought as facilitators that help induce innate immune response but also help achieve the needed activation of T and B cells to induce the adaptive immune response. There are different mechanisms to produce an immune response and some adjuvants can use one or more of these action mechanisms. They can, for example, improve the immunoavailability of the antigen or provide right costimulatory signals to the T and B cells required for proper antigen recognition (Davies, 2010). Some antigens can form a depot at the injection site where they aggregate and hold antigens to release them slowly over a longer period of time (Awate et al., 2013; Wu and Liu, 2021). This enhances the antigen availability by influencing the concentration, place, and time of the antigen disposal (Davies, 2010). This can happen, for example, if the adjuvant has changed the physical properties of the antigen (Wu and Liu, 2021). By aggregating the antigens to a local area, the destruction of circulating antigens by liver clearance is prevented leading to increased and constant stimulation of immune response. Adjuvants such as water-in-oil emulsions and microparticles have been shown to form a depot to produce high and long-lasting antibody titers but it has also been shown that depot formation might not be necessary for the activity of some adjuvants such as alum or MF59 (Awate et al., 2013). While alum has several mechanisms of action, there is some disagreement about whether it acts through depot formation, although alum has been thought to bind the antigen through electrostatic interaction and so improve the antigen uptake and presentation by APCs (Marrack et al., 2009; Davies, 2010; Awate et al., 2013).

Other adjuvants can function through up-regulation of cytokines and chemokines by forming proinflammatory conditions to the injection site enhancing the immune response. As an example, alum, CpG oligodeoxynucleotides (CpG-ODN) and MF59 can induce cytokine production to regulate the immune response (Awate et al., 2013). Proinflammatory conditions and the expression of cytokines, related to for instance migration of immune cells into peripheral tissues, attract more immune cells to the area, and adjuvants can further help activate these cells to produce more cytokines boosting the immune response and antigen presentation. Furthermore, by affecting the local cytokine production, adjuvants can take part in affecting the formation of the specific antibody

isotype (Davies, 2010). The optimal isotype is vital for the removal of pathogen for different antibodies have different abilities to activate and affect different parts of the immune response. For example, some isotypes activate the complement system while some facilitate binding to phagocytes in a different way.

Moreover, adjuvants can absorb antigens onto its surface or otherwise interact with it which can improve the delivery of the antigen to the APCs and enhance the internalization of the antigen (Wu and Liu, 2021). This mechanism also helps to retain the antigen active for a longer time but is especially vital the activation of T and B cells. The activation of the lymphocytes requires two signals: one where the antigen is presented on an MHC molecule and another one through costimulatory molecules. Furthermore, naïve T and B cells usually migrate via blood between secondary lymphoid tissues and only memory and effector lymphocytes can migrate to peripheral tissue during an inflammation (Davies, 2010). For this reason, it is important that the antigen is processed and presented on APCs so that the antigen is also delivered to the secondary lymphoid tissues for antigen presentation and activation of adaptive immune response.

The costimulatory signals needed for lymphocyte activation can be both inhibitory and stimulatory and it is the total quantity of those signals that determines what kind of adaptive response forms (Davies, 2010). Most often, adjuvants are those stimulatory ones signalling that the presented antigens are appropriate to induce the immune response (Davies, 2010). PRRs can help in costimulatory signalling as well as in the antigen presentation. For example, TLRs may affect the formation of TCR ligands affecting the presentation of the antigen (Davies, 2010). They can also guide the adaptive immune response to preferred direction, for instance, toward either Th1 or Th2 response. In addition, adjuvants that work as TLR ligands can enhance the T cell response stating that antigen and adjuvants that are both delivered to the APC can induce a proper antigen presentation and antigen specific adaptive response (Davies, 2010).

Additionally, there are some variables that can affect the adjuvant action and the efficiency of antigen presentation such as particle size, shape, surface chemistry and texture (Awate et al., 2013; Barclay and Petrovsky, 2017). Smaller particles have been shown to be more easily taken into cells by DCs but particles that are too small end up being phagocytosed while particles with similar measurements to known structures enhance DCs' ability to induce T cell activation (Barclay and Petrovsky, 2017). On the other hand, the shape of the molecule can affect the half-life and distribution of the molecule. Surface chemistry can also affect the uptake by DCs for larger particles that have a positive charge on their surface are taken up more easily into the cells. Also, mechanical properties such as rigidity and how well the particle mimics natural structures and surface textures can affect the phagocytosis of adjuvant and/or antigen by macrophages and DCs.

Several adjuvants act through PRRs that recognize the adjuvants as danger signals or the local damage caused by the adjuvants causing the activation of the receptors and cells leading to induced immune response (Davies, 2010). Activated and mature APCs express more MHC and co-stimulatory molecules (Awate et al., 2013) to reinforce antigen procession and presentation which are vital in activating adaptive immune response and further, a proper T cell activation. For example, lipopolysaccharide (LPS), adjuvant system 04 (AS04, which is a trade name for combination of the TLR4 agonist MPL and aluminium salt) and CpG-ODN have been shown to stimulate APC maturation (Awate et al., 2013). After proper maturation and activation, the APCs migrate into draining lymph nodes where they present antigens to B and T cells to produce antibodies or effector T cells (CD8+), respectively.

On the other hand, some adjuvants can create a local tissue damage at the site of injection as opposed to adjuvants that induce the production of proinflammatory cytokines (Awate et al., 2013). The DAMPs activate innate immune response and stimulate adaptive response as well. These events can lead to inflammasome activation through NLR family member. Lastly, adjuvants can also affect the type of antibody production and produce or enhance delayed-type allergy as well as regulate what kind of immune response forms (Wu and Liu, 2021). In other words, adjuvants can affect both the qualitative and the quantitative characteristics of the immune response and its elements which reflect on the efficacy of the vaccine used.

5.2 Adjuvant types

There are several different classes of substances used as adjuvants such as mineral salts, emulsions, liposomes, and microparticles (Awate et al., 2013) but they can also be classified according to their mechanism of action, administration route or physical and chemical properties. Table 1 introduces examples of some adjuvants used in marketed vaccines and lists their mechanism of action and what kind of immune response they activate. Although the action mechanisms have been listed in here, in most cases detailed understanding of why and how they act this way is still mostly unclear and remains to be defined.

Table 1. The action mechanisms of adjuvant examples used in marketed vaccines. Modified from Awate et al. 2013. (Davies, 2010; Awate et al., 2013; O'Hagan et al., 2020; Pulendran et al., 2021; Wu and Liu, 2021)

ADJUVANT ADJUVANT COMPONENTS	ACTION MECHANISMS	ACTIVATED IMMUNE RESPONSE	EXAMPLES OF MARKETED VACCINES
ALUM ALUM ALUMINIUM HYDROXIDE ALUMINIUM PHOSPHATE ALUMINIUM SULFATE	<ul style="list-style-type: none"> - antigen absorption - recruitment of cells (e.g., eosinophils, neutrophils) - local IL-1β secretion promotes antibody production - antigen presentation - independent of TLR signalling - NLRP3 inflammasome activation in DCs and macrophages (relevance is unknown) - enhances adaptive immune response by causing tissue damage 	<ul style="list-style-type: none"> - enhances antibody and Th2 responses - poor Th1 response 	<ul style="list-style-type: none"> - Diphtheria, Tetanus, and Pertussis (DTap) - Hepatitis A - Hepatitis B - Japanese encephalitis (IXIARO®)
MF59 SQUALENE TWEEN 80 SPAN 85	<ul style="list-style-type: none"> - recruitment of cells - local proinflammatory chemokine secretion - antigen uptake and loading in APCs and APC activation - TLR independent MyD88 activation for antibody response - NLRP3 independent ASC activation - stimulation of antigen-specific CD8+ T cells in tissues via RIPK3-dependent pathway - induces transient release of ATP enhancing adaptive immune response 	<ul style="list-style-type: none"> - balanced Th1 and Th2 responses 	<ul style="list-style-type: none"> - influenza vaccine (Fluad®) - H1N1 pandemic vaccines (Focetria® and Celtura®; both are off-market)
AS04 ALUM MPL (TLR4 AGONIST)	<ul style="list-style-type: none"> - TLR4 activation leading to APC activation - local cytokine, chemokine secretion - recruitment of cells - antigen loading onto APCs - activates transcription factor NF-κB 	<ul style="list-style-type: none"> - enhanced antibody and Th1 responses 	<ul style="list-style-type: none"> - HPV (Cervarix™) - HBV (Fendrix®)

AS03 SQUALENE A-TOCOPHEROL TWEEN 80	<ul style="list-style-type: none"> - chemokine secretion locally and in draining lymph nodes - cell recruitment and activation of monocytes and macrophages - antigen loading onto APCs - disruption of lipid metabolism in monocytic cells - enhances upregulation of genes involved in e.g., antigen presentation and processing 	<ul style="list-style-type: none"> - enhanced antibody, CD4+ T cell and T follicular helper cell responses and immune memory 	<ul style="list-style-type: none"> - pandemic flu (e.g., Pandemrix®; now off-market)
AS01 MPL QS-21 LIPOSOMES	<ul style="list-style-type: none"> - local chemokine secretion and cell recruitment - activation of innate immune response via TLR4 - activation of NK (IFN-γ secretion) and CD8+ T cells in draining lymph nodes - aids in monocyte differentiation - activation of caspase 1 in subcapsular sinus macrophages 	<ul style="list-style-type: none"> - enhanced cell-mediated immune response (T cells) and production of antigen-specific antibodies 	<ul style="list-style-type: none"> - Malaria (Mosquirix™) vaccine - Shingles vaccine (Shingrix)
CPG SYNTHETIC DNA	<ul style="list-style-type: none"> - TLR9 activation leading to MyD88 pathway and type I interferon response 	<ul style="list-style-type: none"> - enhanced antibody response - Th1 cell response - stimulation of B cell and NK cell activation 	<ul style="list-style-type: none"> - HBV vaccine (Hepelisav B)
VIROSOMES	<ul style="list-style-type: none"> - binds to APCs and induces receptor mediated endocytosis - antigen presentation to CD4+ and CD8+ T cells 	<ul style="list-style-type: none"> - enhanced antibody and T_k cell responses 	<ul style="list-style-type: none"> - influenza vaccine (Inflexal®) - Hepatitis A vaccine (Epaxal®) both are off market

APC, antigen presenting cell; ASC, apoptosis-associated speck-like protein containing a CARD; DC, dendritic cell; HBV, hepatitis B virus; HPV, human papilloma virus; IFN- γ , interferon-gamma; IgE, immunoglobulin E; IL-1 β , interleukin-1 β ; MyD88, myeloid differentiation factor 88; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NK cell, natural killer cell; NLRP3, NLR family pyrin domain-containing 3; RIPK3, receptor-interacting serine-threonine kinase 3; Th1, T helper 1; Th2, T helper 2; TLR, Toll-like receptor

6. CONCLUSION

The human immune system is a complex network where different components work seamlessly together in various formations to protect the body from different threats. Very much is already known about it and how it works but still some mysteries remain. That is mostly because many pieces are

included in the system, but they all can act differently depending on the threat. Usually, the basic outline of the response is the same, but the outcome can be different and this can be just because of, for example, a few changes in the cytokine profile or the cells responding to the threat.

This can also be seen in the vaccine mediated protection. Some vaccines can produce a long-lasting immunity against a pathogen, but some just last few months if not less. Additionally, there are vaccines that can also protect from a different pathogen or a different strain of the same pathogen while some do not. Furthermore, in most cases, the reason why this happens is a mystery. However, this has not hindered the development of new vaccines and methods to produce vaccines, and this was especially shown in the case of SARS-CoV-2 outbreak. New technological developments and larger investments as well as the increased research could open the door to possible advances in vaccine development against more challenging pathogens and diseases not caused by microbes.

Similarly, this could also be applied to the adjuvants. At first, more simple adjuvants consisting of one or two components were used but now more advanced and complex combinations are produced, although, some of the more recent ones in use or in development are combinations of the ones already in use. Although there is much that is already known, there is also a lot that remains a mystery. Most of the times it is connected to the action mechanism of the adjuvant as well as to the signals and receptors induced in the immune response that are connected to the adjuvant. Although extensive research is done, sometimes the results are somewhat inconclusive or contradict each other which leads to more of a speculation on how the adjuvant works. However, the gained knowledge in immunology has revealed several targets for adjuvant development (Pulendran et al., 2021) that are components of the immune system. These include for example PRRs, molecules regulating immune cell function and molecules affecting intracellular response to PAMPs. Further advances in technology will determine how and how well these adjuvant candidates can be utilised in vaccines.

Nevertheless, while adjuvant development has taken a leap forward, one thing remains the same: the difficulty of defining what an adjuvant is and what can be considered to be an adjuvant. This is also reflected in how the adjuvants are classified. They could be classified in several ways but usually the difficulty comes in that one adjuvant can be put in several boxes. For example, one adjuvant can work through different vaccine administration methods, or act through different action mechanisms that might not be fully understood. Further studies on the action mechanisms of adjuvants and properties they possess could help in defining a good way to classify them which would take in consideration the ones already in use and the ones still in development. Lastly, all the knowledge received from the studies could be beneficial for the development of vaccines and vaccine design as well as help find and bring new adjuvants to be used in vaccines.

7. REFERENCES

- Awate, S., Babiuk, L.A. and Mutwiri, G. (2013) 'Mechanisms of Action of Adjuvants', *Frontiers in Immunology*, 4.
- Barclay, T. and Petrovsky, N. (2017) 'Chapter Seven - Vaccine Adjuvant Nanotechnologies', *Micro- and Nanotechnology in Vaccine Development*, pp. 127-146.
- Bennett, K.M., Rooijackers, S.H.M. and Gorham, R.D. (2017) 'Let's Tie the Knot: Marriage of Complement and Adaptive Immunity in Pathogen Evasion, for Better or Worse', *Frontiers in Microbiology*, 8.
- Burton, D.R. (2002) 'Antibodies, viruses and vaccines', *Nature Reviews Immunology*, 2(9), pp. 706–713.
- Cruse, J.M., Lewis, R.E. and Wang, H. (eds) (2004) '8 - B CELLS, IMMUNOGLOBULIN GENES, AND IMMUNOGLOBULIN STRUCTURE', in *Immunology Guidebook*. San Diego: Academic Press, pp. 277–309.
- Davies, G. (ed.) (2010) *Vaccine adjuvants: methods and protocols*. New York, NY: Humana Press (Springer protocols, 626), pp. 1-11, 103-105, 131-132, 169-170, 287-302.
- Di Pasquale, A., Preiss, S., Tavares Da Silva, F. *et al.* (2015) 'Vaccine Adjuvants: from 1920 to 2015 and Beyond', *Vaccines*, 3(2), pp. 320–343.
- Garçon, N., Chomez, P. and Van Mechelen, M. (2007) 'GlaxoSmithKline Adjuvant Systems in vaccines: concepts, achievements and perspectives', *Expert Review of Vaccines*, 6(5), pp. 723–739.
- Ghattas, M., Dwivedi, G., Lavertu, M. *et al.* (2021) 'Vaccine Technologies and Platforms for Infectious Diseases: Current Progress, Challenges, and Opportunities', *Vaccines*, 9(12).
- Hedman, K., Heikkinen, T., Huovinen, P. *et al.* (eds) (2011) *Mikrobiologia, immunologia ja infektiosairaudet. Kirja 2, Immunologia*. Helsinki: Kustannus Oy Duodecim.
- Janeway, C.A., Travers, P., Walport, M. *et al.* (2001) 'The complement system and innate immunity', *Immunobiology: The Immune System in Health and Disease. 5th edition*. New York: Garland Science.
- Keshavarz-Fathi, M. and Rezaei, N. (2019) 'Chapter 3 - Vaccines, Adjuvants, and Delivery Systems', in Rezaei, N. and Keshavarz-Fathi, M. (eds) *Vaccines for Cancer Immunotherapy*. Academic Press, pp. 45–59.
- Marrack, P., McKee, A.S. and Munks, M.W. (2009) 'Towards an understanding of the adjuvant action of aluminium', *Nature Reviews Immunology*, 9(4), pp. 287–293.
- O'Hagan, D.T. and De Gregorio, E. (2009) 'The path to a successful vaccine adjuvant – “The long and winding road”', *Drug Discovery Today*, 14(11), pp. 541–551.

O'Hagan, D.T., Lodaya, R.N. and Lofano, G. (2020) 'The continued advance of vaccine adjuvants – "we can work it out"', *Seminars in Immunology*, 50.

Pollard, A.J. and Bijker, E.M. (2021) 'A guide to vaccinology: from basic principles to new developments', *Nature Reviews Immunology*, 21(2), pp. 83–100.

Pulendran, B., S. Arunachalam, P. and O'Hagan, D.T. (2021) 'Emerging concepts in the science of vaccine adjuvants', *Nature Reviews Drug Discovery*, 20(6), pp. 454–475.

Varkila, K. and Hurme, M. (1992) 'Auttaja-T-solujen alaryhmien merkitys infektioitaudeissa ja allergiassa', *Vol. 108 (21)*, pp. 1849–1856.

Walsh, E.R. and Bolland, S. (2014) 'Chapter 6 - B Cells: Development, Differentiation, and Regulation by Fcγ Receptor IIB in the Humoral Immune Response', in Ackerman, M.E. and Nimmerjahn, F. (eds) *Antibody Fc*. Boston: Academic Press, pp. 115–129.

Wu, Z. and Liu, K. (2021) 'Overview of vaccine adjuvants', *Medicine in Drug Discovery*, 11.