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Alum as an adjuvant for human vaccination

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In field of vaccination, to get better immune response, alum is the sole adjuvant approved for human vaccination. Alum is able to induce a good antibody (Th2) response, but it is not effective to stimulate cellular (Th1) immune response, which is so important for protection against many pathogens. Although alum has been used extensively in vaccines for over 70 years, the exact mechanism by which they enhance immune response have not been fully elucidated, which is important for better knowledge of the adjuvants and immune response. In addition alum has the potential to cause severe local and systemic side-effects including sterile abscesses, redness, sub-cutaneous nodules, and some allergic responses, although fortunately most of the more serious side-effects are relatively rare. There is also community concern regarding the possible role of aluminium in neurodegenerative disease such as Alzheimer's disease. This paper reviews the importance of alum adjuvant in human vaccination, explores future directions of adjuvants development and examines some of the impediments and barriers to development and registration of new human adjuvants.

Key words : Alum, Human vaccination

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INTRODUCTION

Adjuvant is derived from latin word adjuvare means to aid. Adjuvant added to vaccine to stimulate the immune system is response to the target antigen but do not in themselves improve the immunity (Gupta and Siber, 1995; Kenney and Edelman, 2004). Aluminium based mineral salts (alum) are the most widely used adjuvants for human vaccination.

The use of adjuvant first introduced by Ramon in 1925, who demonstrated that immune responses of diphtheria and tetanus toxoid could be enhanced by injection of other compounds such as agar, tapioca, lecithin, starch oil, saponin, and bread crumbs (Brewer *et al.,* 1996). The selection of appropriate adjuvant has to be made based on the type of immune response desired or that is necessary for providing protection.

Alum adjuvant:

Alum is a salt with the combination of an alkali metal such as sodium, pottasium, ammonium and a trivalent metal such as aluminium, iron and chromium. Alum can be used in the form of potash alum, soda alum, ammonium alum, chrome alum, selanate containing alum, aluminium sulphate alum, in which potassium aluminium sulphate or potash alum, hydrated potassium aluminium sulphate with the formula KAl $(SO_4)_2.12H_2O$ is the most common form that has been used in food processing also.

Alum was first introduced as an adjuvant in 1926 and continues to be the only FDA approved adjuvant for routine human vaccination in US (Ulanova *et al.,* 2010). It has been used widely and proven effective for enhancing humoral immune responses. Alum is a weak adjuvant for mediating cellular immunity and is associated with generating the production of immunoglobulin E associated with allergic reactions (Brewer *et al.,* 1999; Gupta, 1998; Schyms, 2000).

Adjuvant and adaptive immune response:

Adjuvants are essential for enhancing and directing the adaptive immune response to vaccine antigens.This response is mediated by two main types of lymphocyte and T cells. Upon activation by cytokines, B cells differentiate in to memory B cells (long-lived antigen-specific B cells) or plasma B cells (effector B cells that secrete large quantities of antibodies). Most antigens activate B cells using activated T helper cells

(Th), primarily Th1 and Th2 cells.

Th1 cells secrete IFN-y which activates macrophages and induces the production of opsonizing antibodies by B cells.

The Th1 response leads mainly to a cell mediated immunity (cellular response) which protects against intracellular pathogens. Natural killer (Nk) cells are also activated by the Th1 response.

Th2 cells secrete cytokines, including IL-4, which induces B cells to make neutralizing antibodies. Th2 cells generally induce a humoral (antibody) response.

IL-12 is a hetrodimeric cytokines that stimulates the production of interferon gamma from T-cells and natural killer cells, and also induces differentiation of Th1 helper cells.IL-12 is an initiator of cell- mediated immunity. The magnitude and type of Th response to a vaccine can be greatly modulated through the use of adjuvants. For almost 70 years, aluminium salts (referred to as 'alum' have been the only adjuvant in use in human vaccines.

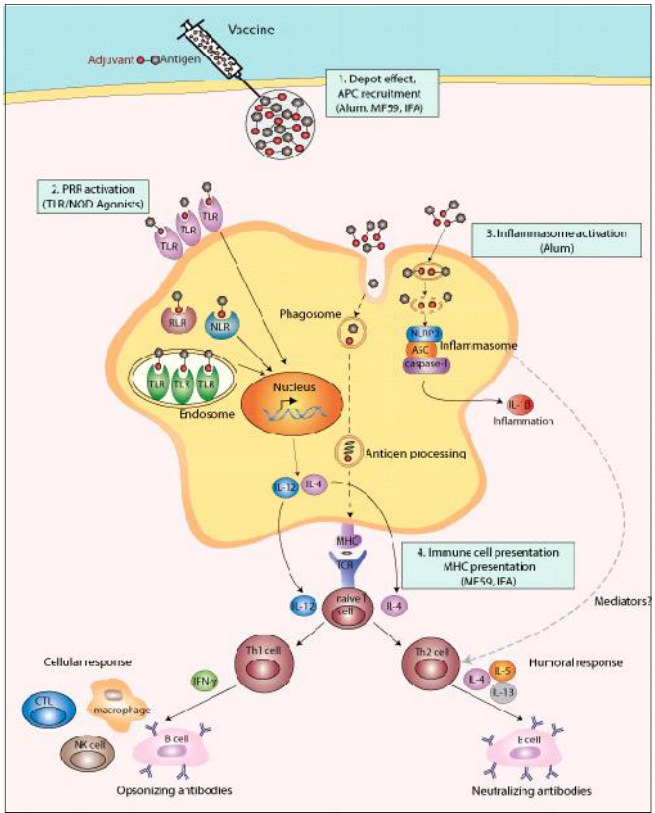

Fig. 1 :

Mechanism of alum adjuvant:

Although alum has been used extensively in vaccines for over 70 years, the exact mechanism by which they enhance immune responses have not been fully elucidated. Proposed mechanism include:

- Enhancing the uptake of associated antigen in to APCs.
- Forming a depot in macrophages present in muscle.
- Causing a local inflammatory response due to necrosis at the site possibly resulting in the release of inflammatory cytokines and activation of APCs (Mark *et al.*, 1995; Krewski *et al.*, 2000; HoginEsch, 2002; Brewer, 2006).

Alum as a combined adjuvant increases the affinity of antigen:

Many researchers have investigated the combination of various adjuvant systems as a means for obtaining synergistic enhancements in immune responses.

Alum (Particulate system) when combined with many immunostimulatory adjuvants such as Lipid A derivative, CpG and IL-12 increases the affinity of many antigen like Herpes simplex virus, Hepatitis B surface antigen, Respiratory syncytial virus, HIV-1 gp 120, Malaria antigen (Alving, 1993; Sparwasser *et al.*, 1999; Becker, 2005; Hancock *et al.*, 2000; Su *et al.*, 2003). Alum is a safe and effective method for enhancing humoral and antibody responses to surface bound or co-entrapped antigen in human clinical trials (Rao and alving, 2000).

Particulate adjuvant (alum) have the capability to bind antigens to form multi-molecular aggregates which will encourage uptake by APCs (Leroux-Roels, 2010).

Other adjuvants essentially ligands for pattern recognition receptors (PRR), act by inducing the innate immunity predominantly targeting the APCs and consequently influencing the adaptive immune response. Members of nearly all of the PRR families are potential targets for adjuvants. PRR ligands are classical adjuvants induce strong Th2 response with little Th1 response, the current challenge is to develop adjuvants which induce a strong Th1 bias important for vaccines against hepatitis, flu, malaria and HIV.

PRR activation stimulates the production of proinflammatory cytokines/ chemokines and type IFNs that increase the host's ability to eliminate the pathogen. Thus, the incorporation of pathogens associated molecular patterns (PAMPs) in vaccine formulation can improve and accelerate the induction of vaccine-specific responses. A number of these agoinst are now in clinical or late pre-clinical, stages of development for hepatitis and human papillomavirus vaccines (Mbow, 2010). When used in combination with alum, the immune response can be biased towards a Th1 response (Didierlaurent, 2009).

The adsorption of IL-12 on alum has also been investigated to enhance Th1 type immune responses to several antigens (Hancock *et al.*, 2000; Becker, 2005; Su *et al.*, 2003).

Alum and emulsions:

Adjuvants may exert their effects through different

mechanism. Adjuvants, such as alum and emulsion (*e.g.* MF59), function as delivery systems by generating depots that trap antigens at the injection site, providing slow release in order to continue the stimulation of the immune system. Alum is the most commonly used adjuvant in human vaccination. It is found in numerous vaccines, including Dtp, HPV and hepatitis vaccines (Krewski *et al.*, 2004). Alum provokes a strong Th2 response, but is rather ineffective against pathogens that require Th-1 cell mediated immunity. Alum induces the immune response by a depot effect and activation of APCs. Recently the NLRP3 inflammasome has been linked to the immunostimulatory properties of alum (Li, 2008) although its role in adjuvant-induced antibody responses remains controversial.

Emulsions (either oil in water or water in oil), such as Freund's incomplete adjuvant (IFA) and MF59 can trigger depot generation and induction of MHC responses. IFA induces a predominantly Th2 biased response with some Th1 cellular response. MF59 is a potent stimulator of both cellular (Th1) and humoral (Th2) immune responses (Ott, 1995). However, the precise mode of action of emulsion-based adjuvants is their potential to induce auto immunity.

Some alum containing vaccines:

Alum is used in many subunit vaccines as an adjuvant to enhance the body's response to immunogens. When alum is used to enhance a vaccine, it is classified as an adjuvant or supplementary contributing agent. Adjuvant containing aluminium have been shown to make some vaccines last longer and appear to often produce more antibodies against disease. Relatively common vaccines licensed in the United States and typically administrated to children include:

DTP (Diphtheria-Tetanus-Pertussis):

In this vaccine

Diphtheria:

Diphtheria is an upper respiratory tract illness caused by *Corynebacterium diphtheriae*, a facultative anaerobic Gram-positive bacterium. It is characterized by breathing problems, paralysis, heart failure and even death.

Pertussis:

Pertussis commonly known as whooping cough is a highly contagious respiratory tract infection characterized by typical cough with whoop. Pertussis is a highly contagious bacterial disease caused by *Borcletella pertussis*. Complications in pertussis are pneumonia, encephalitis (due to lack of oxygen) and occasionally death, especially infants.

Tetanus:

Tetanus is a serious disease caused by anaerobic bacteria *Clostridium tetani* that cause painful frightening of the muscles, usually all over the body. It can lead to 'locking' of the jaw so the victim cannot open his mouth or swallow. Tetanus leads to death in about 1 in 10 cases.

DTaP — (Diphtheria-Tetanus- a cellular Pertussis):

DTaP, a cell free vaccine prepared from purified antigenic components of cell free microorganism, carrying less risk than whole cell preparations.

Hepatitis A:

Hepatitis A vaccine is a vaccine against the hepatitis A virus. Hepatitis A is a disease of the liver caused by hepatitis A virus. It is characterized by jaundice.

Hepatitis B:

Hepatitis B vaccine is a vaccine against the hepatitis b virus (HBV) which infects the liver and causes an inflammation called hepatitis. It is characterized by lifelong infection of the liver, liver cancer, liver failure and death.

Human papilloma virus (HPV):

The human papilloma virus (HPV) vaccine prevent infections with certain species of human papilloma virus associated with the development of cervical cancer, genital warts and some less common cancers.

HPV Vaccines-Gardasil and cervarix:

Both vaccines protect against the two HPV types (HPV-16 and HPV-18) that cause 70 per cent of the cervical cancer 80 per cent of anal cancers, they also cause most HPV induced oral cancers.

Gardasil also protect against the two HPV types (HPV-6 and HPV-11) that cause 90 per cent of genital warts. Both vaccines have been shown to prevent potentially precancerous lesions of the cervix, gardasil has been shown to prevent potential precursors to anal, vulvar, vaginal and penile cancers.

Rabies :

Rabies is a viral disease that, cause acute encephalitis (Inflammation of the brain) in warm blooded animals. It is zoonotic (means it can be transmitted from one species to another) rabies virus infects the central nervous system, ultimately causing disease in the brain and death.

Early stage symptoms of rabies are malaise, headache and fever progressing to acute pain, violent movements, uncontrolled excitement, depression and hydrophobia. Finally the patient may experience periods of mania and lethargy eventually leading to coma. The primary cause of death is usually respiratory insufficiency. Rabies can be prevented by rabies vaccine both in humans and other animals.

Safety of alum containing vaccines:

There has been a search for alum adjuvants will continue to be used for many years due to their good track records of safety, low cost and adjuvanticity with a variety of antigens.

The adverse effects of alum adjuvants are rare, however, local reactions such as redness, swelling and/or tenderness at the site of injection are not infrequent, the pain and redness known to occur more readily with these vaccines may indicate that shot is doing its job. More severe local reactions such as large areas of swelling, sterile abscesses, subcutaneous (sc) nodules (some lumps under the skin, some of which have inflammation in the tissue) and allergic responses are much less common.

A recent review of the evidence of adverse events, after exposure to alum containing vaccines against diphtheria, tetanus, and pertussis (DTP) found no evidence that the salts cause any serious or long lasting adverse events.

Alum exposure:

Alum is exposed in a large quantity in our daily use. Alum is approved by the U.S. food and drug administration as a food additive, the most common form, potassium aluminium sulphate or potash alum, is one form that has been used in food processing. Another sodium aluminium sulphate is an ingredient in commercially produced baking powder, potash alum is an astringent/styptic and antiseptic, for this reason it can be used as a natural deodorant by inhibiting the growth of the bacteria responsible for body odor. Its astringent /styptic properties are often employed after shaving and to reduce bleeding in minor cuts and abrasions, nosebleeds and hemorrhoids. It is frequently used topically and internally in traditional systems of medicine including ayurveda and traditional chinese medicine. It is also used in tanning of leather.

However, historically some individuals with kidney failure who were exposed to large quantities of aluminium salts when undergoing dialysis –developed serious neurological effects.

Various government agencies establish guidelines for exposure to potentially toxic substances. These guidelines are called "minimal risk levels''-the maximum amount that one can be exposed to over-time usually on a daily-basis-without expected harm. The U.S agency for toxic substances and disease registry (ATSDR) estimated these levels for infants taking into account the amount of alum a child would eat or receive by injections of vaccines. The body burden of alum from both source is below the minimal risk level except transiently following vaccination; since 50-70 per cent of injected alum is excreted with in 24 hrs, this is believed to have no negative effect (Keith *et al.,* 2002).

Conclusion:

Despite an explosion of knowledge regarding immune function over recent decades, we remain almost totally reliant for human adjuvants on alum based compounds whose activity was first discovered over 75 years ago. They are appropriate adjuvants for vaccine that confer protection by inducing antibodies via the induction of type 2 immune responses, but they do not induce cytoxic T cells and cell mediated immunity.

The mechanism by which alum adjuvants selectively enhance the immune response is poorly understood. A recent review of alum containing vaccines found no evidence that salts cause any serious or long lasting adverse events.

Besides vaccination alum is exposed in a large quantity, in many other uses such as food additive, as an astringent/ typtic and antiseptic, it is also used in tanning of leather. Exposure of large quantities of aluminium salts may cause kidney failure and neurodegenerative disease, so various government agencies establish guidelines for exposure to potentially toxic substances. These guidelines are called "minimal risk levels''-the maximum amounts that one can be exposed to over- time usually on a daily-basis without expected harm.

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