



Immunological survey of plant derived materials used as adjuvant for specific protein antigen: Review

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Abstract

The role for vaccine adjuvants in case of veterinary and human vaccines has been a matter of great interest for the last so many years. Till now, only one adjuvant i.e. alum that are approved by Food and drug administration (FDA) for human use as well as for research purpose. Now a day, researchers worked with some plant based immunostimulators pertaining to determined humoral and cell mediated immune response against specific protein antigen. The only drawback for alum which enhanced only humoral response and poorly elicited cell mediated immunity. In an effort to search for those adjuvants that are derived from various medicinal plants and showed minimal side effects including toxicity. In this review, we focused on plant derived molecules or candidate used as adjuvant against specific protein antigen.

Keywords: vaccine; adjuvant; alum; plant

Introduction

An adjuvant is an agent that excites the immune system by enhancing the retaliation to a vaccine. The term adjuvants come from the Latin word *adjuvare* which means support (to help) and is defined as a substance which prolongs, stimulates and intensifies the quality of certain immune responses when integrated into vaccines formation. Their response is persuaded by the antigen alone and it may also provide the hazardous signal, the immune system needs in order to react to the antigen as it would do to an active infection ^[1]. The immune system has two definite and imbricate mechanisms with which to fight conquering organisms, the antibody-mediated defense system (humoral immunity) and the cell-mediated defense system (cellular immunity) ^[2]. The term therapeutic plants which include varied types of plants used in analgesics and some of these plants including its fruit, seed etc. have a number of medicinal activities or properties ^[3, 5]. There are many adjuvants having familiar use worldwide, including aluminum salts, oils and virosomes ^[6]. Adjuvants in immunology are used to alter or enhance the effects of a vaccine by stimulating the immune system to respond to the vaccine more strenuously, and thus providing increased immunity to a specific ailment. Adjuvants are also used in the production of agglutinin from inoculated animals. It can also act as an irritant, which captures and boosts the body's immune response ^[7]. They are needed to improve routing and adaptive immune responses to antigens. This reaction is mediated by two main types of Lymphocytes, B and T lymphocytes. These have various mechanisms of action of adjuvant is related with immunomodulation, which allude to the capability of many adjuvants to modify the system of cytokines ^[8].

In the late 1980's, immunologists start focusing on T-cells and B-cells, the drivers of adaptive immunity. In other words, B-cells are responsible to make antibodies that released into the blood and fight against various extracellular infections. Since the mid-1990s, scientists

including researchers have made many new discoveries in the field of innate immunity. So, most of the researchers have found that vaccine adjuvants work by eliciting or generating early, innate immune responses. These approaches have led to identification and recognition of many promising adjuvant candidates that targeting both innate as well as adaptive immunity ^[9, 10].

In 1996, Jules Hoffman and his colleagues reported that a receptor called as Toll, essential for fruit flies to raise or climb up an immune response against fungus. Simultaneously, Janeway and his post-doc student, Ruslan Medzhitov, reported a protein called as human Toll, recently called as Toll-like receptor 4 (TLR4) and this receptor observed on the cell surface and played a crucial role in innate and adaptive immunity ^[11, 12]. Thereafter Hoffman's discovery, Medzhitov and Janeway published scientific paper in 1997 showing that this human toll also turned on proteins known to be tangled in co-stimulating T-cells and B-cells.

Finally, scientists identified various molecules that are present on innate immune cells where adjuvants can directly target and should be able to induce both innate as well as adaptive immunity. So, researchers agree that adjuvants may directly act on particular types of immune responses, enroll particular populations of immune cells and play an indispensable role in the ability of innate immune cells pertaining to shape the adaptive immune response (T and B cells). Increasing understanding of how adjuvants work to stimulate particular immune responses (innate as well as adaptive) and play an important role in the development of new and improved vaccines.

According to the World Health Organization (WHO), around three-fourth of the world population, depends upon conventional remedies, generally herbs for health care of its people. In fact, herbs and plants not only give food and shade but also serve to cure various afflictions. Numerous metabolites are present in plant extracts which are responsible for inducing activity of adjuvants against some

antigens. In modern medicines, drugs developed from natural or plant based products have been used to treat infectious diseases, cancer, hypertension and inflammation [13].

Mechanism of Action of Adjuvants

Many molecules interact with the properties of the adjuvants whose biological heterogeneity obstructs the classification and division into distinct groups that makes it easier to select a desired purpose of adjuvants. Depending on the routes of administration, immune responses and antigen, the mechanism of action of adjuvants can be portrayed into numerous ways-

- Many therapeutic effects of compounds derived from plants like colchicine, curcumin, resveratrol and quercetin and phytochemicals present in them like flavonoids, saponins, diterpenoids, alkaloids etc. have shown an immunomodulatory effect that influences the immune system and the mechanism of action is somehow associated with the immunomodulation, which refers to the medications used for regulating and normalizing the immune system. In general, in presence of an immunogen, only the adjuvants associated with the immunomodulatory agents can stimulate an adjuvant effect, which further results in directly regulating the production of cytokines and immune system. Therefore, some inhibitory responses are produced simultaneously, like TH1 cell response that enhances the fixation of components of complement system in relation with the hypersensitivity reaction associated with interferon gamma (IFN- γ) and TH2 cell response that highly results in the production of secretory antibodies, IL-4, IL-5 and immunoglobulin IgE. It also neutralizes the effect of bacterial toxins [14, 15].
- The mechanism requires a single type of adjuvant that allows the antigen to be processed in cytosol which induces the peptides with the MHC-I complex and later on presents itself on the surface of cell for the induction of cytotoxic T-lymphocyte (CTL) responses [16].
- In contrast, depot effect is the most wide recognized mechanism of adjuvants. The antigen is trapped and slowly released at the site of injection that produces an increase in antibody titers by ensuring the initiation of the immune system response [16]. The adjuvants in gel form like aluminum hydroxide or in form of emulsions like complete Freund's adjuvant (water-in-oil emulsion) increases its immunobiologically half-life.

Plant Based Adjuvants

During the last two decades several vaccine industries have been established in the country to cater domestic and international needs on vaccine products. Due to unsuitability of conventional attenuated vaccines as safe healthcare products, the world is looking for subunit vaccines as safer alternatives and development of suitable and complimentary adjuvants is mandatory for evolving such new efficacious, nontoxic vaccines. Several major human infectious diseases and cancers do not have effective vaccines and there is a major unmet need for developing effective vaccines through co development of nontoxic adjuvants. This is particularly relevant given the global scenario that all the vaccine adjuvants approved for human use are toxic and multi ingredient formulae. Even though the world vaccine

manufacturers realized this emergency and started putting earnest effort to develop adjuvants, India has not made any strides in this direction so far. Indian vaccine industries are badly in need of adjuvant expertise. The concept of plant based molecules is to generate powerful and safe adjuvants for vaccines against number of infectious diseases.

Infectious diseases are considered to be the hot topic in the area of research. In an effort to treat current infectious diseases are still under threat, different types of therapies are used that include drugs or vaccines or both. Recently, the whole world was facing the problem related to various pathogenic microorganisms and responsible for causing disease in animal and human. A lot of efforts should be taken in order to reduce the burden of various infectious diseases. Therefore, there is an urgent need to improve or develop next generation of vaccines, in particular against infectious diseases. Majority of cases, use prophylactic type of vaccines can help to control the incidence rate of infectious diseases. Although number of animal and human diseases have been controlled or reduced in most parts of the world, re-emergence of some diseases like polio that still remains a threat. In addition, number of diseases like rabies, influenza and some relatively reported new challenges like Ebola that can locally cause high mortality rate in various European countries and most of these cases is also reported in India as well. In this article, we discussed about some medicinal plants and tried to use as an adjuvant for vaccine antigen against infectious diseases. In other words, we discuss about the prospects of medicinal plant products as alternative platform or criteria for the screening and development of next-generation of vaccine adjuvants that can be a cost-effective source for people in developing countries.

Traditionally, Ayurvedic plants used as medicine for the treatment of many infectious diseases for more than 3000 years and increasingly lot of evidences that are reported and showed that administration of plant based candidates in comparison with standard (western medicine therapies) and it may reduce side effects or/and improve the health of animal/human against infectious diseases. Recently, medicinal plants derivatives like glycosides, saponins and polysaccharides isolated and purified through various sophisticated and well established techniques i.e. high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC) etc. Now, researchers have received considerable attention due to their effect on measurable therapeutic properties and low toxicity, in both experimental (i.e. animal and human) systems. Here is a figure showing plants based adjuvants in vaccine immunogenicity and some functions of adjuvants.

- *Andrographis paniculata*: It is an erect annual herb, commonly known as green chiretta belonging to the family *Acanthaceae*. It is mainly found in Asian countries like India, Nepal, China, Sri Lanka etc. It has potential antioxidant, anti-ulcer, anti-stimulatory properties which make it a very important immunostimulatory agent. The extracts of the plants leaves help to keep the liver healthy and promotes a better digestion also. The herb contains diterpenoids, flavonoids, polyphenols as major bioactive components. Immunomodulatory effect of andrographolide and 14-deoxy-11, 12-didehydroandrographolide show immunostimulatory

- activity by increasing the production of interleukin and lymphocytes^[17].
- Water-soluble biopolymeric fraction i.e. BOS 2000 isolated and purified from the gum resin exudates of *Boswellia serrata* belonging to the family *Burseraceae* and is generally observed in the traditional Ayurvedic system of medicine for the treatment of various inflammatory diseases. In search of a potent plant based vaccine adjuvant i.e. BOS 2000, water-soluble biopolymeric fraction was evaluated for immunological activity against HBsAg. As per the literature, BOS 2000 is a potent enhancer of antigen-specific (Th1 and Th2) immune responses in comparison to alum with Th2 limitation and is a promising adjuvant for vaccine applications^[18].
 - *Butea frondosa* (palas; family *Fabaceae*), medicinal plant commonly reported all over India and showed various medicinal properties i.e. flowers (fever, thirst and diarrhoea); decoction of its bark (bleeding piles); seeds paste (skin diseases, edema and eye diseases). As per the literature, immunoadjuvant activity of *Butea frondosa* using HBsAg as specific protein antigen pertaining to determined its T cell-mediated immunity (i.e. complex interactions between the lymphoid and phagocytic cells) that played an important role in eliminating intracellular infections and responsible for enhancing the lymphocyte proliferative response. In literature, aqueous leaves extract could significantly enhanced the T cell response i.e. cell mediated immunity against specific protein antigen^[19].
 - Plant based adjuvant candidate i.e. RLJ-NE-299A from the roots of *Picrorhiza kurroa* and showed its immunostimulatory and adjuvant activity as well. In literature, adjuvant candidate RLJ-NE-299A up-regulated the expression of Th1 (cell mediated immunity) cytokines IL-12, IFN-gamma, TNF alpha and Th2 (humoral immunity) cytokine IL-4 in lymph node cell culture supernatant containing HBsAg. In short, RLJ-NE-299A has strong potential to increase both cell mediated and humoral immune responses^[20].
 - *Phyllanthus emblica*: Also known as Indian gooseberry belonging to the family *Phyllanthaceae* plays a very important role in stimulating or boosting up our immunity. The fruit of this plant contains Gallic acid quercetin, kaemferol, emblicanin, flavonoids, glucosides, vitamin C, tannins which makes it a very powerful immunostimulant and antioxidant in nature. All parts of the plant have immunoadjuvant properties. It is used in the treatment of inflammation, diarrhoea, jaundice etc^[21].
 - *Panax ginseng*: Commonly known as Korean ginseng, belonging to the family of *Araliaceae*, is a perennial herb cultivated majorly in the Northern part of Asia, has some major immunoadjuvant properties. It contains a complex mixture of steroidal saponins called ginsenosides and other compounds. It promotes vitality and stamina in people of any age, strengthens the immune system function, supports and maintain cellular health and also acts as stress busters^[22].
 - *Echinacea purpurea*: Commonly known as purple coneflower, is a North American species of flowering plant in the sunflower family has reportedly shown immunostimulatory properties, especially in cold and cough condition. The extracts of the plants have also shown positive effect in patients suffering from anxiety and depression. Studies are going on and the plant has also shown to have antibacterial, antiviral and anti-cancer properties. The secondary metabolite derived from this plant includes alkamides, caffeic acid derivatives, polysaccharides, and glycoprotein which are pharmacologically active^[23].
 - Capsaicin: It is a type of plant derived immuno adjuvant, obtained from chilli peppers. It has been used for a long time as traditional medicine in different regions to use it for joint and muscle pain. The research on capsaicin resulted in the discovery of transient receptor potential channel vanilloid subfamily member 1 (TRPV1), which is the direct target of capsaicin^[24].
 - Triterpenoid saponins, specially, Quill A saponins (deriving a purified form of adjuvant), derived from a plant named *Quillaja saponaria*, has been used in veterinary vaccines as it has proven to be an adjuvant with a strong potential that can directly get associated to induce the production of cytokines. The production of IG2a and IG2b antibodies against antigens and the stimulation of cytokines (IL-2 and INF- γ) induces a powerful TH1 response generated by the purified saponin Quil A adjuvant named QS-21. The unique feature of Quill A, saponin and its purified adjuvant QS-21 has the ability to generate immune responses by producing cytotoxic T- lymphocytes against the exogenous antigens and also reduces the toxicity which stimulates the Th2 response. QS-21 is made of a core triterpene attached to an aldehyde and two sugar chains, out of which one out of three is acylated by an ester bond-lipophilic aliphatic acid bound fructose. The presence of fatty acids indicates the interactions between saponins and membrane cholesterol that promotes hemolysis, whereas, a lipophilic side chain that initiates a CTL response which works against exogenous proteins^[25].

Conclusion

In short, plant-based vaccine adjuvant may offer a range of advantages over traditional vaccine production and delivery systems. A promising approach is the production of vaccines containing plant-based adjuvant. Two major obstacles have been encountered in developing vaccines. Firstly, the expression level in terms of foreign antigens are reported tends to be low and secondly, co-expression of an adjuvant mainly plant derived may be required pertaining to facilitate an appropriate type of immune response. Plant based adjuvants are emerging as suitable candidates and increasing efforts are being made to discover potential suitable adjuvants. Plant based material may be attractive candidates for use as vaccine adjuvants that stimulate both Th1 and Th2 immune responses.

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