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A review on herbal immunoadjuvant

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Abstract

The world population relies upon traditional remedies (mainly herbs) for the health care of its people. In fact, herbs and/or plants are the oldest friends of mankind. There are biological or synthetic substances that can stimulate suppress or modulate any aspect of the immune system including both adaptive and innate arms of the immune system. The present paper enumerates the herbal immunoadjuvant.

Key-Words: Herbs, Immunity, Immunoadjuvants

Introduction

According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people. In fact, herbs and/or plants are the oldest friends of mankind. They not only provided food and shelter but also served to cure different ailments. Herbal medicine, sometimes called traditional or natural medicine, has always existed in one way or another in different cultures and civilizations, such as Ayurvedic (India), Egyptian, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani-Tibb (south Asia).

In the early nineteenth century, the advent of modern medicine saw a rapid decline in botanical medicinal use. Vaccination, the discovery of antibiotics and improvements in medical technology all contributed to this demise. Today, plant-derived medicines are classified as complementary and alternative medicines (CAM) and are regulated by the Therapeutic Goods Administration (TGA) in Australia and by the Dietary Health and Supplement Education Act (DHSEA) under the Federal Drug Administration (FDA) in the USA¹⁻³

Vaccines are one of the most successful interventions for infectious diseases. However, major challenges remain in vaccine design, including improving their efficacy significantly and developing new vaccines for emerging diseases. Current vaccines typically include an antigen or live attenuated microorganism, an adjuvant to enhance the immune response, and a delivery system to target delivery to the right location.

An adjuvant is an agent that stimulates the immune system, increasing the response to a vaccine, while not having any specific antigenic effect. Adjuvants perform one or more of three main functions. (i) They provide a “depot” for the antigen for slow release; (ii) they facilitate targeting of the antigen to immune cells and enhance phagocytosis, and (iii) they modulate and enhance the type of immune response induced by the antigen alone⁵⁻⁸ Adjuvants may also provide the danger signal the immune system needs in order to respond to the antigen as it would to an active infection⁹. Thus, adjuvants play a significant role on every aspect of the immune response.

Immunity

This may be defined as the body’s ability to identify and resist large numbers of infectious and potentially harmful microorganisms, enabling the body to prevent or resist diseases and inhibit organ and tissue damage. The immune system is not confined to any one part of the body. Immune stem cells, formed in the bone marrow, may remain in the bone marrow until maturation or migrate to different body sites for maturation. Subsequently, most immune cells circulate throughout the body, exerting specific effects. The immune system has two distinct but overlapping mechanisms with which to fight invading organisms, the antibody-mediated defense system (humoral immunity) and the cell-mediated defense system (cellular immunity).¹⁰

Immune systems

The basic architecture of the immune system is multilayered, with defenses on several levels. Most obvious and primary is the skin: the first barrier against infection.

Another is physiological, where conditions like the temperature and pH of the body provide inappropriate living conditions for foreign organisms. Once

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pathogens have successfully entered the body, they are addressed by the innate and/or the acquired or adaptive immune system. Both systems consist of a multitude of cells and molecules that interact in a complex manner to detect and eliminate pathogens. Detection and elimination depend upon chemical bonding: surfaces of immune system cells are covered with various receptors, some of which chemically bind to pathogens, while others bind to other immune system cells or molecules to enable the complex signaling system that mediates the immune response.¹¹

Immunomodulators

These are biological or synthetic substances that can stimulate, suppress or modulate any aspect of the immune system including both adaptive and innate arms of the immune system.

Classification of immunomodulators

Clinically, immunomodulators can be classified into the following three categories: **Immunoadjuvants** are used to enhance the efficacy of vaccines and therefore could be considered specific immune stimulants. Immunoadjuvants hold the promise of being the true modulators of the immune response. It has been proposed that they be exploited as selectors between cellular and humoral helper T1 (Th1) and helper T2 cells (Th2), immunoprotective, immunodestructive, and reagenic [immunoglobulin E (IgE)] versus IgG type immune responses posing a real challenge to vaccine designers.¹²

Immunostimulants are inherently non-specific as they are envisaged as enhancements to a body's resistance to infection. They can act through innate as well as adaptive

immune responses. In healthy individuals, the immunostimulants are expected to serve as prophylactic and promoter agents, i.e., as immunopotentiators, by enhancing the basic level of immune response. In the individual with impairment of immune response, they are expected to act as immunotherapeutic agents.¹³

Immunosuppressants are a structurally and functionally heterogeneous group of drugs, which are often concomitantly administered in combination regimens to treat various types of organ transplant rejection and autoimmune diseases.¹⁴

Immune response mechanisms¹⁵⁻²³

A physiological immune response begins with the antigen presenting cell (APC). This is the crucial step of the activation of the immune system. The best APCs responsible for activation of helper T cells, killer T cells and B cells are dendritic cells (DCs). Immature DCs are found under the skin and mucous membranes where they sample surrounding for possible pathogens

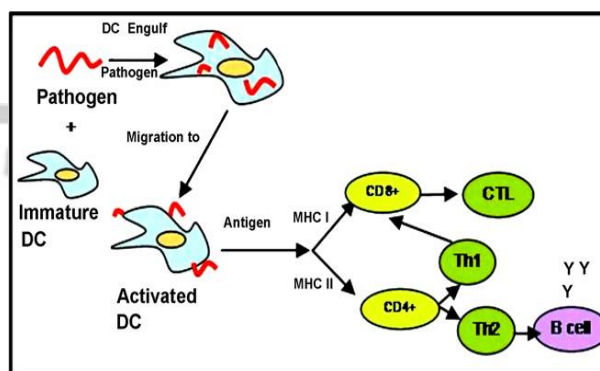


Fig. 1: Mechanism of immune response

through pattern recognition receptors. After detecting pathogen, these cells engulf it via phagocytosis and pinocytosis and migrate to lymph nodes where they become mature. Once inside the DC, pathogens are degraded into small fragments that are further expressed at their surface where they can be presented to T cells and B cells (Fig. 1). After the T cells and B cells become activated, they generate a cascade of events that lead to attack the disease. There are two antigen presenting pathways within DCs that lead to the major histocompatibility complex (MHC) molecules. These molecules bind peptide fragments from pathogens and display them on the DC surface for recognition by T cells. One of these pathways is endogenous, which involves presentation of MHC I molecules to CD8+ T cells. The CD8+ T cells activated by DCs presenting antigens can kill infected cells directly by activating cytotoxic T lymphocytes (CTLs). On the other hand, the exogenous pathway involves presentation of MHC II molecules to CD4+ T cells. A subset of the activated CD4+ T cells, known as helper T cells, Th1 and Th2, are responsible for cell-mediated immunity (CMI) and humoral immunity, respectively. T cells of the immune system which generate CMI or a Th1 response require the antigen to be processed and presented on the surface of an antigen presenting cell to stimulate T cells. B cells are responsible for the humoral or Th2 response, and they recognize antigens through B cell receptors and can secrete antibodies in response. Most current vaccine designs generate an acquired immune response but do not stimulate the Th1 pathway. An immune response of the Th2-type is characterized by the production of cytokines such as IL-4, IL-5, IL-10 and IL-13 producing elevated IgG1 and IgE antibody isotypes. Th1-type responses are characterized by the production of the cytokines IFN- γ and TNF- α , antibodies of the IgG2 isotype and are usually associated with CMI including activated macrophages and delayed-type hypersensitivity.. Immune responses of the Th1 type are directed more

towards intracellular pathogens and are necessary for clearance of many viruses, some bacteria (e.g., *Mycobacterium tuberculosis*) and anti-tumor effects, whereas a Th2 response is generally sufficient for the neutralization of toxins, viruses, and bacterial adhesion. Further, CD4+ cells can be classified as Th17 or Treg. For efficacious vaccines, it is essential to induce the appropriate immune response is essential for vaccine efficacy. For example, in the BALB/c model of leishmaniasis, a Th2-biased immune response does not afford protection and the mice are unable to clear the infection. In another example, the currently used Bacille Calmette Guerin (BCG) vaccine for tuberculosis is ineffective in preventing disease because it is unable to redirect a pre-existing immune response (due to previous infection) to a protective, Th1 dominant immune response. Similarly, current vaccines against feline infectious peritonitis viruses are ineffective because they are biased towards enhancing humoral immunity, which has been shown to exacerbate the disease, whereas a CMI or regulatory response would be protective. As mentioned before, DCs are critical components of innate immunity that affect the acquired immune response (and their activation is a powerful tool to manipulate the immune system. DCs are present in an immature state where they cannot stimulate T cells, but the presence of an antigen causes the DCs to mature and mobilize. Therefore vaccines that can stimulate DC maturation are promising in order to obtain a more balanced immune response and to increase the efficacy of vaccines. Harnessing both immune system pathways and facilitating immunomodulation to obtain an unbiased immune response (i.e., Th0, which is a balance between Th1 and Th2) is key to effective vaccine treatments against infectious agents

Limitations of current vaccines²⁴⁻³⁰

Current vaccine designs do not target the DC system. DCs can readily stimulate T cells and can operate at mucosal surfaces, where early protection is needed in many infections, while existing vaccines are weak stimulators of T cells. T-cell activation is only guaranteed by repeated encounters with persistent low levels of antigens. Therefore therapeutic strategies based on modulating the immune response may significantly expand treatment options and circumvent the problem of rapid emergence of resistance. The major advantage of infectious disease therapies based on immunomodulation is that it harnesses a system that has evolved and is continuously evolving to protect against microorganism-related diseases. While both the innate immune system and antimicrobial agents show rapid onset of action, modulators

of innate immunity are not likely to develop resistance because they do not disable a specific microbial target and their mechanisms of action involve multiple effector cells. Vaccinology, including adjuvant design, has focused mainly on parenteral routes of administration. However, most pathogens enter through mucosal routes such as oral, nasal or genital and parenteral vaccines alone do not typically produce mucosal immune responses. The difference between the mucosal mode of entry of most pathogens versus the parenteral route of administration of most vaccines leads to stimulation of production of different isotype antibodies. Current vaccines elicit mainly IgG isotype antibodies, in contrast to natural infections that elicit a wide range of antibody isotypes. Therefore the protection afforded by the vaccines is not as long-lasting. Immunization of one mucosal surface also sensitizes other remote mucosal surfaces because of a common mucosal immune system. However, mucosal delivery of vaccines poses several challenges. Most protein antigens are poor immunogens when administered mucosally and may induce immunological tolerance instead. There have been several intranasal vaccines that have been developed to counter infectious diseases such as diphtheria and pneumonic plague. These vaccines are more effective when adjuvanted with either microbially derived components or synthetic polymers and are usually inhaled in particulate form. Some of these intranasal vaccines induce cell-mediated immunity, leading to protection. On the other hand, oral administration provides access to the largest immunological organ of the body, the intestine. Digestive degradation following oral administration causes large and repeated doses of killed microbe or peptide antigens to be required. Therefore potentially new solutions to this problem are imperative, and a U.S. National Research Council (NRC) panel recently addressed grand challenges in this area. The panel's main recommendations to improve vaccine design include: (1) Research to identify good delivery mechanisms and (2) identification of potential molecular targets for targeting DCs and modulating innate immunity without undesired side effects. The NRC report identified the determination of how to elicit protective non-IgG responses and simulate the mucosal response, and devising strategies to target DCs and optimize antigen delivery to DCs as the most likely approaches to improve vaccinations and combat infectious diseases.

Immunomodulation by plant-derived medicines

Experimental evidence has shown that the development of immune responses following infection or vaccination can be influenced by plant-derived

medicines. The ability to modulate immune function offers many advantages from maintaining health through immunity to stimulating or suppressing beneficial or deleterious immune responses. Plant medicines could mediate such effects through targeted modulation of key cellular and molecular interactions of antigen-presenting cells (e.g. dendritic cells), T-lymphocytes and B-lymphocytes as illustrated by the paradigm in Fig. 1. Identification of such immunomodulatory properties will be crucial in the discovery of novel, clinically-relevant compounds for augmenting existing immunotherapy or vaccination practices. Perhaps more importantly, immunomodulatory plant-derived medicines may be critical in developing regions of the world – where burden of disease is high and access to medical care limited – as a low cost alternative to achieve a better health status through enhanced protective immunity. It is imperative therefore to elucidate the mechanism(s) by which plant-derived medicines modulate immune responses. In this context, the important functions of dendritic cells (DCs), B-lymphocytes and T-lymphocytes are discussed.

Modulation of dendritic cell activity

Dendritic cells (DCs) are recognised as specialised ‘professional’ antigen-presenting cells capable of initiating and directing immune responses following infection. Dependent on the pathogenic ‘signals’ received by DCs, the immune system can be triggered to respond in a TH1 or TH2 restricted manner³⁰⁻³¹. As such, DCs are often described as critical regulators of immunity that operate at the interface between the innate and adaptive immune systems. Dendritic cells that reside in peripheral lymphoid tissues become activated following encounter and phagocytosis of antigen through engagement of cell-surface receptors (e.g. Toll-like receptors [TLRs] and CD14) with pathogenic molecules such as bacterial lipopolysaccharide (LPS)³²⁻³³. Activated DCs interact with antigen-specific CD4⁺ T-lymphocytes and migrate to the infected site where the expression of co-stimulatory molecules on the cell surface is upregulated (e.g. CD80, CD40, CD86, and CTLA-4) to stimulate appropriate effector T-lymphocyte function³⁴⁻³⁵. Given the integral role for DCs in the initiation of immune responses, experimental approaches based on the design of novel therapeutics that exploit DC function may provide effective alternatives to current immunotherapies. Indeed, emerging vaccine candidates that modulate the activity of DCs are of particular interest where certain types of immune responses have proven difficult to generate with existing vaccines³¹.

The biological activities of plant-derived medicines are therefore dependent on the species, purified components and experimental conditions used. The identification of immunosuppressive drugs is becoming increasingly important in the context of the rising incidence of chronic diseases such as autoimmunity, allergy and cancer. Plant compounds that are able to down-regulate DC-specific immune parameters may provide an alternate new class of pharmaceuticals. Treatment of human DCs with *Tripterygium wilfordii* saponins in vitro resulted in 75% lower CD80 expression that was postulated to be IL-10 dependent, suggesting a mechanism of action for this preparation. Furthermore, the suppression of LPS-induced MHC Class II, CD80 and CD86 expression on DCs by the polyacetylene compound, faltarindiol (from *Notopterygium incisum*) was due to inhibition of the transcription factor, NF- κ B [44]. Differential effects on human DC maturation in vitro was observed for various *Echinacea purpurea* extracts, with increased expression of CD83 by the root and flower extracts whereas whole plant and stem/leaf extracts reduced this expression³⁶⁻⁴³.

Herbal component	Interact with specific-antigen	Action
Polysaccharides from <i>Astragalus mongholicus</i>	CD11c and MHC Class II molecules in vitro	enhanced DC antigen presentation capacity
flavonoid-rich fraction of <i>Alchornea cordifolia</i>	increased HLA-DR, CD40, CD80 and CD86 expression	Maturation of human and murine DCs
volatile oil rich extracts of <i>Amomi</i> seed	activation of CD86 but not CD80	murine bone marrow-derived DCs
steroidal saponin M4 <i>Ginseng</i>	higher T-cell stimulatory capacity in a mixed lymphocyte reaction	to immature DCs in vitro
saponin-rich <i>Panax notoginseng</i> extract	TLR ligands such as LPS, CpG and poly (I:C)	to attenuate the activation of murine DCs

Modulation of B-lymphocyte function

B-lymphocytes are critical for the induction of effective antibody based immunity following pathogen

challenge. Current vaccines have been developed to exploit the exquisite specificity of antibody secreted by B-lymphocytes to confer protection from diseases such as diphtheria, tetanus, pertussis, hepatitis, measles, Haemophilus influenzae type b(Hib), and pneumococcal and meningococcal infections [47–49]. Plant-derived medicines that modulate B-lymphocyte effector functions may be a useful tool in the maintenance of protective immunity in resource-poor settings where access to vaccines is limited. Moreover, the demonstrated activity of plant drugs delivered mucosally offers significant advantages over the conventional parenteral routes of immunisation in these regions.⁴⁴⁻⁴⁶

Parenteral administration

The parenteral administration of plant-derived medicines has been a convenient method to identify bioactive properties on host specific immune responses. Even in the absence of antigen, plant drugs are able to modulate adaptive immune responses.

Plant-derived medicines	Administration	Immune responses ⁵¹⁻⁵⁹
fruit-derived monoterpenes, limonene, perillidic acid and carvone	intraperitoneally (ip) to mice	enhance the anti-sheep red blood cell (SRBC) antibody response up to 10-fold above untreated mice
vegetable-derived isothiocyanates or a Tridax procumbens extract,	immunisation (ip)	increased B-cell proliferation by an elevated SRBC plaque-forming cell (PFC) response
total (T)-ginseng preparation	guinea pigs immunised with a porcine parvovirus (PPV) vaccine	serum haemagglutination inhibition (HI) antibody titres six times higher than guinea pigs immunised with PPV alone
purified Astragalus polysaccharides	rabbit HI antibody titre	inactivated rabbit hemorrhagic disease (RHD) vaccine up to 49 days post-immunisation compared to a non-adjuvanted vaccine
Polygala	mice	producing a four-

senega vaccine	immunised subcutaneously with a combined ovalbumin (OVA)-	fold rise in serum anti-OVA IgG compared to control mice
Bupleurum chinense or Glycyrrhiza uralensis	mice immunised with OVA	stimulating serum OVA-specific IgG, IgG1 and IgG2b titres up to 16-fold equivalent to the human vaccine adjuvant, alum and was attributed to its saponin content
Saponins isolated from Anemone raddeana (ARS)	mice	enhanced the OVA-specific IgG, IgG1 and IgG2b levels in mice

Mucosal administration

The adjuvant activity of plant-derived medicines is particularly important in the context of mucosal vaccines. Since infections occur predominately through the mucosal surface, new-generation vaccines that elicit protective immunity following mucosal administration will be critical. Using this approach, the oral administration of a phenolic-rich extract of *Mangifera indica* to mice augmented the serum anti-SRBC HA titre almost 20-fold above control mice. More recently, these authors reported that the serum anti-influenza titres of mice were substantially elevated following immunisation with a combined intranasal influenza vaccine containing saponins derived from *P. tenuifolia*. The effects of *P. tenuifolia* in this study was attributed to enhanced APC activity and that the combined vaccine preparation may protect from antigen degradation events, allowing a slow and sustained exposure to the immune system. It could be proposed that such activity might ultimately provide heightened and prolonged T and/or B-lymphocyte activation resulting in the reported increased antibody response detected. Similarly, the anti-influenza IgG response of mice orally administered with *Pinellia ternata*-derived *Pinellia* tuber for 16 days was elevated above control mice.⁴⁷⁻⁴⁹

Modulation of T-lymphocyte function

Pathogenic molecules are presented to T-lymphocytes by dendritic cells in peripheral lymphoid tissues and, depending on the nature of the signals received via T-cell receptor (TCR) engagement, co-stimulation and

the cytokine milieu, produce TH 1 or TH2-based immune responses. Although critical to the success of vaccination, these responses can also be deleterious when unregulated in the context of allergy and autoimmune diseases. The presence and function of CD4+ T-lymphocyte populations such as regulatory T cells (Treg), TH17 and TH9 cells all contribute to the balance between health and disease. Equally important is the development of CD8+ T-lymphocytes that act to destroy and remove virally-infected cells and tumours. The discovery of plant-derived medicines that modulate CD4+ and CD8+ T-lymphocyte function will therefore be an important class of novel therapeutic agents.⁵⁰

Plant-derived medicines that modulate CD4+ T-lymphocytes

Data from in vitro studies have documented the effects of plant derived medicines on CD4+ T-lymphocyte function.

Plant-derived medicines	CD4+ T-lymphocytes ⁶⁰⁻⁶⁵
Bupleurum falcatum-derived saikosaponin-d	enhance the proliferative response of concanavalin A (ConA)-stimulated mouse spleen cells associated with higher IL-2 production
Sho-seiryu-to following incubation with ConA treated mouse splenocytes	reduction in IL-4 mRNA
alkylamides derived from <i>E. angustifolia</i> and <i>E. purpurea</i>	increased the level of the NF- κ B in LPS and PMA-stimulated human Jurk at T-lymphocytes had no such effect on unstimulated cells
<i>C. sinensis</i> & alkaloid containing extracts of <i>Tylophora indica</i>	treatment of ConA stimulated mouse splenic CD4+ T-lymphocytes resulted in abrogated IL-2 levels.
<i>T. wilfordii</i> hook F, triptolide	elicit this activity, and could inhibit peripheral CD4+ T-lymphocytes but increased CD8+ T-lymphocyte numbers in Peyer's patches of mice in a collagen-induced mouse arthritis model of inflammation

Plant-derived medicines that modulate CD8+T-lymphocyte function

The cytotoxic CD8+ T-lymphocyte response is particularly important for the protection against virally-infected cells and tumour cells.

Plant-derived medicines	Modulate CD8+T-lymphocyte ⁶⁶⁻⁶⁸
Oral administration of mice with <i>S. cerevisiae</i> -derived β -glucan	increased the levels of CD8+ and CD4+ intraepithelial lymphocytes (IELs) compared with control mice with increased IFN γ mRNA in IELs
Lentinan, the β -glucan purified from <i>Lentinus edodes</i>	antigen-specific CTL production in vivo and in vitro through increased IL-2 responsiveness of CD8+ T-lymphocytes
oral treatment of mice with a water-extract of <i>Dok Din Daeng</i>	increased the T cell proliferative response by 31% compared to control cells in vitro, supporting earlier results using the related <i>A. indica</i> seed extracts

Conclusion

Evidence from the scientific literature supports the use of plant-derived medicines to stimulate immune function. Characterisation of these preparations in terms of biological activity and bioactive components will promote the utility of such preparations in the future. While the potential for use of plant-derived medicines should not be underestimated, the cellular and molecular mechanisms of action need to be clearly defined. One major limitation appears to be the lack of congruence in research strategy and experimental design, with numerous approaches implemented in examination of the biological effects. The choice of plant-derived medicine, whether this is a whole formula, extract or purified component, the level of endotoxin and method of administration are all key questions that influence biological activity. In addition, the dose and timing of administration also needs to be evaluated extensively. In summary, the biological properties of plant-derived are increasingly being appreciated by the scientific community. The immune modulating activity of these preparations has provided the impetus for further characterisation and in some cases, potential mainstream utilisation as part of an integrated healthcare system.

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