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Immunostimulants: Concepts, Types and Functions

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Abstract

The proper functioning of human immune system is essential for organism survival against infectious, toxic and oncogenic agents. The concept of immunomodulation was proposed by Edward Jenner, while working on polio vaccine in 1796. A brawny, fine-functioning immune system is the keystone of excellent health. Immune replies are the consequence of an effectual interaction among innate (natural and non-specific) and acquired (adaptive and specific) components of the immune system. Inequity or failure of the immune systems is connected with a variety of chronic illness counting allergies, autoimmune diseases, cancers and furthers. Immunomodulators are natural or synthetic materials that regulate the immune system and induce innate and adaptive defense mechanisms. These substances are classified into two types, immunostimulants and immunosuppressants. Immunostimulants can enhance body's resistance against various infections through increasing the basal levels of immune response. These agents could increase the oxidative activity of neutrophils, augment engulfment activity of phagocytic cells and stimulate cytotoxic cells as necessary defense mechanisms. The researchers classified the immunostimulants using their origin and mode of action such as bacterial products, complex carbohydrates, vaccines (antigens and adjuvants), cytokines, immunoenhancing drugs, nutritional factors, animal extracts, and plant extracts. The link between immune system with diet, exercise, sleep, stress, microbial exposure, alcohol, water, hygiene are found to influence the immune response to a greater extent. Immunostimulants and some immunity enhancing nutrients improve the functioning of the immune system. In addition, some preventive measures such as healthy diet or proper nutrition, moderate exercise, sound sleep, drinking adequate water, subclinical exposure to microbes and managing stress, good hygiene, are altogether capable of boosting the immune system. In this review, the concepts, types and functions of immunostimulants will be described as a therapeutic approach against different diseases.

Keywords: Immunomodulators, Immunostimulants, Adjuvant, Mechanism, Immune system.

Introduction

Humans and other vertebrates reside in a world that is occupied by a huge range of pathogenic microbes and toxic substances that menace normal homeostasis; and immunity is a specialized form of host defense mechanism that works particularly in relation to the causes and prevention of diseases[1]. Manifestation of disease due to the pathogen depends on its virulence and capability of the immune system; and to achieve resistance against disease, the most important is strengthening the immune system[2]. If the immune system fails become under or over active, or hits the wrong target it can vent a variety of adverse consequences. Under-activity of the immune system lead to loss the defensive mechanism against infections; extreme immune failure results HIV disease, certain cancer, etc. whereas over-activity can lead to autoimmune diseases, including arthritis, inflammatory bowel disease, inflammatory lung disease, connective tissue disease, autoimmune endocrine diseases, multiple sclerosis, etc. So, proper understanding and strictly regulating the immune system has become mandatory. Two main compounds are able to enhance immune responses including adjuvants and immunostimulants. An adjuvant is a substance combined with an antigen for increasing its immune response, but an immunostimulant can induce the immune response without injection with an antigen[3]. There are several types of stimulants with different mechanisms and functions such as bacterial products, complex carbohydrates (*e.g.*, glucans, schizophyllan, scleroglucan, lentinan, statolon, bestatin, acemannan), vaccines, immunoenhancing drugs (*e.g.*, Levamisole, Isoprinosine, Fluoroquindone, Avridine, Polyribonucleotides), nutritional factors (*e.g.*, vitamins, carotenoids, lipids, trace elements, selenium), animal extracts (*e.g.*, chitosan from shrimp), cytokines (*e.g.*, macrophage activating factor, interferon, interleukin-2, tumor necrosis factor), and plant extracts (*e.g.*, Lectins, mitogens such as phytohemagglutinin, concanavalin A) [4]. Two main approaches were determined to evaluate the efficiency of an immunostimulant such as *in vivo* protection against pathogens, and *in vitro assay* of cellular and humoral immune mechanisms. *In vitro* tests should be performed before *in vivo* experiments to clarify the basic mechanisms responsible for the protection. *In vitro* immunostimulant evaluation is usually based on some parameters such as serum lysozyme, complement, total leucocyte count, monocyte/lymphocyte/granulocyte count, antibody titers, phagocytosis, respiratory burst and leucocyte proliferation[4]. Immunomodulation can be either specific or non-specific. Specific immunomodulation is limited to a single antigen such as vaccination, whereas non-specific immunomodulation leads to a further change in immune response both in innate and adaptive immunity causing altered host reactivity to many various antigens[5].

Immune system

The immune system consist of a complex network of specialized cells, tissues, molecules and biological processes within an organism that watches out the continually to protect it against attacks by foreign antigens or invaders (basically microbes-infection causing organisms such as bacteria, viruses, parasites, and fungi or any injury, and disease) [6]. The different organs of human immune system are shown in Figure 1. Some of the potentially infectious agents includes: (a) Viruses, which are sub-microscopic non-living entities that replicates only inside the host cells (living organism) and often results in serious diseases. Examples include influenza virus, human immunodeficiency virus (HIV), herpes simplex virus (HSV, which can cause cold sores or genital ulcers), a newly discovered virus named coronavirus (causes infection in the upper respiratory tract). (b) Bacteria are single celled microbes capable of causing disease when get entry into the body through water, air, soil and also through physical contact. Examples include Staphylococcus and Streptococcus that cause acute infections such as abscesses and sore throats, Escherichia, Salmonella that cause food poisoning and Mycobacteria that cause chronic infections such as tuberculosis and leprosy. (c) Fungi, eukaryotic, nonphototrophic organisms with rigid cell walls, they can be unicellular or multicellular. Examples include Aspergillus that causes allergic disease, Candida that causes thrush, Cryptococcus that causes meningitis and meningo-encephalitis in patients with HIV infection and AIDS. (d) Parasites, which are eukaryotic organisms that live off other organisms, or host, to survive. Some are them are single-celled protozoa that cause diseases for example, malaria; others are large, multicellular organisms (metazoa) example, worms that can be seen with the naked eye. In order to prevent disease, the immune system must able to scan, recognize and attack the foreign invaders by distinguishing self from non-self substances[2]. Self-molecules are those components that belongs to an organism's body which the immune system can distinct from foreign substances. Autoimmunity is an immune response in opposed to its own healthy cells and tissues, which may lead to various diseases[7]. Non-self-molecules are those recognized components that do not belong to an organism's body, they are foreign invaders. One example of non-self-molecules is antigens that cause the immune system to promote the generation of antibodies against it and then combine specifically with them to induce an immune response[8].

Innate and adaptive immunity

Defence against infection is divided into two main forms namely innate immunity and adaptive immunity. Its components are shown in Figure 2. Some of the differences between innate and adaptive immunity are shown in Table 1.

Innate immunity

Innate immunity regarded as the first line of defense from both external and internal attack, also known as natural or native immunity. It is a nonspecific and antigen-independent defensive mechanism which responds immediately and within minutes or hours of meeting an antigen[9]. Its host defense mechanisms are encoded as their mature form by the germ-line gene of the host[1]. This type of immune response is lack in memory to recognize the pathogen, if the same pathogen invades for second time as they cannot generate that immunologic memory. Innate immunity consists of two major components: Humoral (include complement cells) and Cellular (includes neutrophils, macrophages, adnatural killer cells).

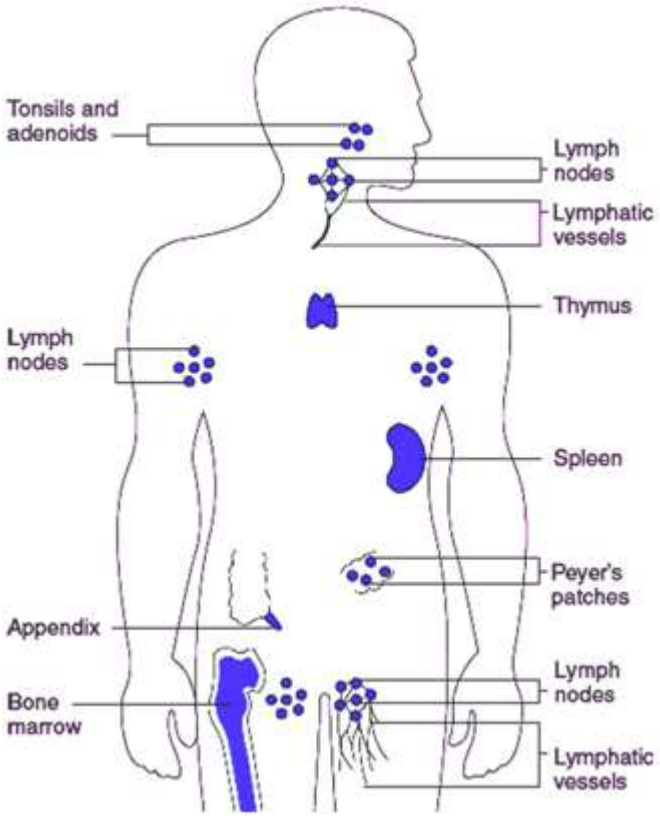

Figure 1: Organs of the human immune system

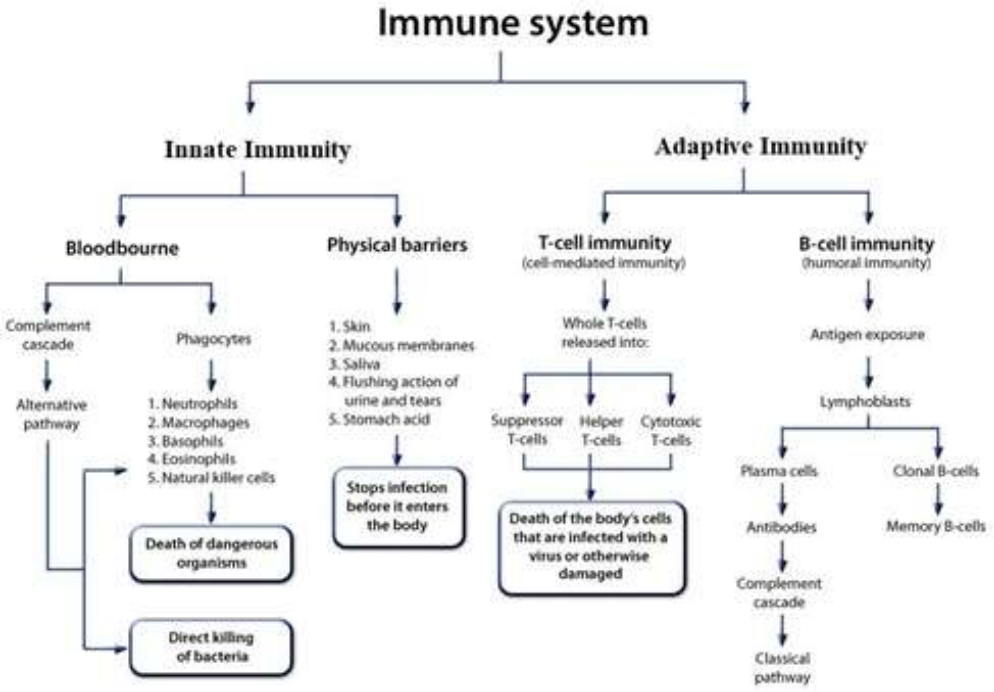

Figure 2: Flowchart of basic components of human Immune system

The defensive barriers of innate immunity are of four types:

- Anatomical barriers e.g., skin and mucous membrane, the epithelial cell layers offers tight junction so there is tight cell to cell contact, the mucus layers over the respiratory, gastrointestinal and genitourinary tract, and when foreign particles are inhaled, the mucus layer get contaminated which are constantly discarded by the epithelial cilia.
- Physiological barriers e.g., temperature, low pH and chemical mediators
- Endocytic and phagocytic cells (neutrophils, macrophages), dendritic cells, natural killer (NK) cells and other innate lymphoid
- Inflammatory barriers e.g., a series of events occurs in inflammation process that plays an important role to destroy or inactivate microbes.

Adaptive immunity

Adaptive immunity is also known as specific or acquired immunity which means the resistance acquired by human during their lifetime. It is antigen-dependent and antigen-specific defensive mechanism and, thus delays the time of the antigen to get expose and to produce the maximal response. The advantage of adaptive immunity is their capacity to generate memory which permits the host to elicit a more rapid, stronger and efficient immune response against consecutive exposure to the antigen[10]. This type of immunity provides the basis for effectual immunization facing infectious diseases. The two major components of adaptive immunity are Humoral (comprises of antibodies formed by B lymphocytes) and Cellular (mediated by T lymphocytes). B lymphocytes and T lymphocytes are two kind of lymphocytes found in this type of immunity that impart long lasting immunity against specific antigens by proliferating into memory cells. Lymphocytes are generated from the bone marrow and the type that mature in bone marrow turns into B lymphocytes whereas the type that leave the bone marrow and migrate to thymus gland get mature into T lymphocytes and based on 'cluster of differentiation' (CD) molecules on their surface they acquire certain genetic and immune surface characteristics which determines their different functions[11]. B lymphocytes are responsible for formation of specific antibodies by differentiating into plasma cells while T lymphocytes get activated in presence of appropriate antigens presented by macrophages like APC and Histocompatibility Complex (MHC). The function of B lymphocytes are like military intelligence system, they find out the target and organise defensive action, while T lymphocyte perform like soldiers, they destroy the invading substance identified by the intelligence system i.e. B lymphocytes[12]. Antigen specific receptors are encoded by genes that are assembled by somatic rearrangement of germ-line gene to form intact T cell receptor (TCR) and immunoglobulin (B cell antigen receptor; Ig) genes. Millions of different antigen receptors are formed from the collection of a few hundred germ-line-encoded gene elements assembly of antigen receptors, each of which are potentially unique and antigen specific[1]. The advantage of this diverseness of receptors helps adaptive immunity to identify any kind of pathogen[13]. They are of two types i.e., naturally acquired adaptive immunity and artificially acquired adaptive immunity.

Naturally acquired adaptive immunity: In naturally acquired active adaptive immunity, antigens enter the body naturally then the bodies develop antibodies and specialized lymphocytes whereas in naturally acquired passive adaptive immunity, antibodies passes from mother to foetus/infant through placenta/mother's milk. Naturally acquired active adaptive immunity lives longer than naturally acquired passive adaptive immunity.

Artificially acquired adaptive immunity: In artificially acquired active adaptive immunity, antigens are introduced into the body through the use of vaccines then the bodies generate antibodies and specialized lymphocytes against it whereas in artificially acquired passive adaptive immunity, preformed antibodies in immune serum are introduced into the body by injection. Artificially acquired active adaptive immunity lives longer than artificially acquired passive adaptive immunity but, when there is very less time to develop active immunity then passive type is more effective as it can prevent the infection in any stage and its process is rapid.

Table 1: Differences between innate and adaptive immunity

Feature	Innate Immunity	Adaptive Immunity
Cells involved	Dendritic leukocyte, Natural killer cells, Mast cell, Granulocytes/ Macrophages,Basophils, etc	Killer CD8+ T-cells, Helper CD4+ T-cells, B-cells, Antigenpresenting cells, etc.
Moleculesinvolved	Cytokines, Complement cells, Interferon, Acute phase reactants/ proteins	Antibodies, Cytokines
Receptors	Germline encoded No somatic rearrangement Non-clonal distribution	Encoded in gene segments Somatic rearrangement necessary Clonal distribution
Action time	Immediate effector activation	Delayed effector activation
Response	Rapidly occurs (0-6 h ours)	Occurs over days to weeks
Order ofdefense	It is the first line of defense ofimmune system	Action against pathogens that are able to evade or overcome innateimmune defense
Immunological memory	None	Confer Immunological memory
Types ofImmune response	Inflammation, Complement mediated killing, Phagocytosis	Antibodies generation, microbial destruction by Helper T cells andCytotoxic T cells
Subsequentexposure	Immune response does not get alter on repeated exposure	Immune response get improves with subsequent exposure
Reason behind immune evasion	Caused by pathogenicvirulence	Caused by mutation of the recognized antigen
Allery or hypersensitivity reaction	None	Immediate and delay hypersentivity
Potency	Lower	Higher
Physioanatomicalcal barriers	Skin, Mucous membranes,Temp, pH, chemicals, etc	Lymph nodes, spleen, mucosal associated lymphoid tissue
Functions	(a) Recruiting immune cells to site of infection; (b) Activationof complement cascade to identify antigens; (c) Identification & removal of foreign substances present in organs, tissues, blood and lymph; (d) Activation of adaptive immune system through antigen presentation; (e) Acting as physical & chemical barrier toinfectious agents.	(a) Recognition of specific "non- self" antigens during the process of antigen presentation; (b) Generation of responses that are tailored to maximally eliminate specific pathogens or infected cells; (c) Development of immunological memory, through memory B cells andmemory T cells.

Functioning of immune system

The immune system comprised of cells and proteins that uphold the body from the foreign invaders[14]. These cells emanate from the pluripotent stem cells of bone marrow. Of the two pathways - (I) the myeloid pathway, in presence of IL-3, becomes excited giving rise to the production of platelets, erythrocytes, monocytes and granulocytes. (II) the lymphoid pathway, in presence of IL-7, becomes excited giving rise to the production of innate and adaptive lymph cells (Lymphocytes). The pathways distinction relies on the chemical signals in the surrounding area[15,16]. When pathogens invade the host body, innate immunity provides the first line of defense. Leukocytes like dendritic cells, monocytes, neutrophils, macrophages, eosinophils, mast cells, are allowed by the pattern recognition receptor (PRRs) to detect and react rapidly towards a large population pathogen which is structurally similar, known as pathogen associated molecular patterns (PAMPs). An example of these is the components of bacterial cell wall like lipopolysaccharides (LPS) and double-stranded ribonucleic acid[17]. The binding of PRPs with PAMPs triggers the release of cellular messenger called cytokines (e.g. interleukin) and causes inflammatory reaction. Inflammation leads to vasodilation, increased vascular permeability and cellular infiltration due to which the microbial cells get destroyed. Other cells, like natural killers are the critical members of innate immunity asthey able to pursue and kill a vast number of pathogens along with malignant cells. All the cells of immune system are capable to suppress or induce inflammation by communicating one another over direct cell contact or through generation of cytokines. Again a complex systems of proteins known as the complement system also induces inflammatory response that aid to fight infection. This type of immunity also aid to remove dead cells or foreign substances from different organs, blood and lymph[13]. The adaptive response takes over when the innate immune response becomes ineffective to eliminate pathogen. In adaptive immunity at first antigen presenting cell (APC) like macrophages and dendritic cells recognizes, engulfs and process the antigen; and displays the specific part of antigen

on its surface then present it to T- cells. T-cells receptors are there that bind with the specific antigenic sites and triggers proliferation and differentiation processes in lymphoid tissues[2]. There are two classes of T-cells namely, helper T-cells and Cytotoxic T-cells which can be discriminate by their presence of some molecules on their surface like CD4+ and CD8+ respectively. T-helper cells aid the immune response in recognition of antigen and then activate other T and B-cells by secreting cytokines whereas Cytotoxic T-cells aid the immune response by killing pathogen infected cells or tumor cells. One other class of T-cells, known as suppressive T-cells are able to secret suppressive cytokines that can inhibit the actions of other T-cells. Antigen binding and helper T-cell can trigger the differentiation process of B-cells into plasma cells and secret antibodies which circulate in the blood and causes destruction or inactivation of the antigen[18].

Immunostimulants

Immunostimulants (or immune stimulants) are biologically active substances obtained from natural or synthetic sources with different chemical characteristics and mechanism of action that modulate the immune system of host to increase resistance against various infections [19]. They interact with specific receptors and cellular components of innate and adaptive response to modulate the immune response. They used during suppressed immunity condition like cancer disease, AIDS, SARS etc. to improve the host's resistance[19].

Concept of immunostimulant

Immunostimulants known as immunostimulators are attractive substances that activate the immune system of humans and animals for prevention of diseases and improvement of the body's natural resistance to various viral and bacterial infections. These biologically active substances are the products derived from natural sources or synthetically made with different chemical properties and mechanisms of action. In general, immunostimulants induce synthesis of specific antibodies and cytokines for treatment of infectious diseases. Two major groups of immunostimulants contain a) specific immunostimulants acting as antigen for stimulation of immune responses (*e.g.,* vaccines), and b) non-specific immunostimulants without antigenic properties enhancing immune responses to other antigens (*e.g*., adjuvants and non-specific immunostimulators). Moreover, immunostimulants were classified based on their origin and mode of action[20].

Functions of immunostimulants

Immunostimulants activate different elements of the immune system in humans and animals. They develop the non-specific immunotherapy and immunoprevention by stimulating the major factors of the immune system including phagocytosis, properdin and complement systems, protective secretory IgA antibodies, α- and γ-interferon release, T- and B-lymphocytes, synthesis of specific antibodies and cytokines, and synthesis of pulmonary surfactant. There are several reasons for using the immunostimulants in the control of various infectious diseases including: a) antibiotic resistance of the bacteria; b) allergic reactions to antibiotics; c) immunosuppressive effects of antibiotics; and d) Poor effects of the antibiotics in viral infections[19]. Figure 3 shows some types of immunostimulants and their general functions.

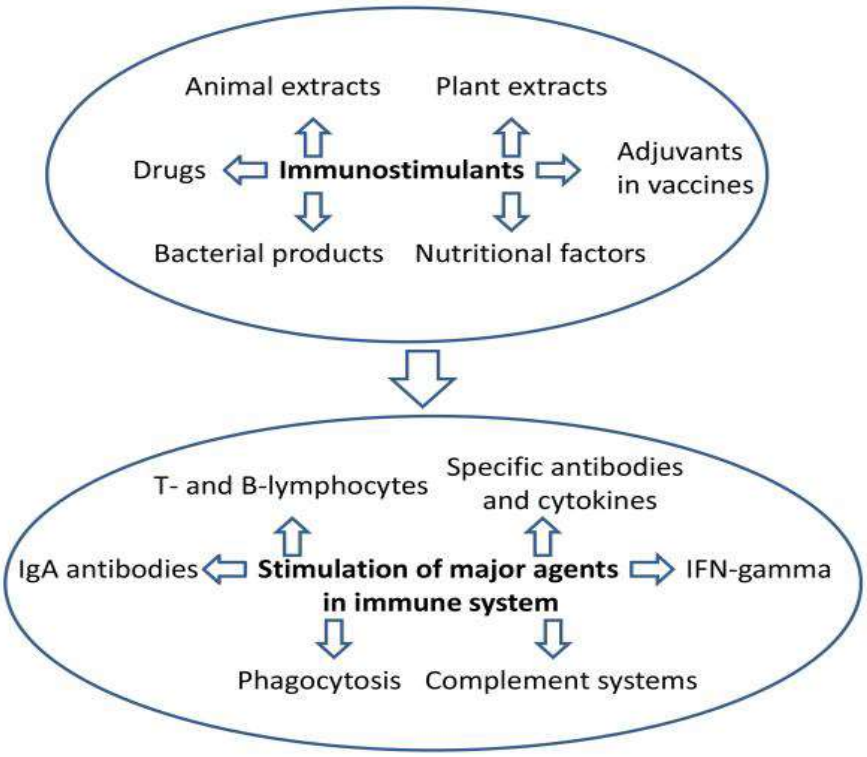

Figure 3: Schematic representation of some types of immunostimulants and their general functions

Types of Immunostimulants

For simplification, we divided the types of immunostimulants as 7 groups such as bacterial products, complex carbohydrates, vaccines (antigens and adjuvants), cytokines, immunoenhancing drugs, plant extracts, and animal extracts as mentioned below:

Immunostimulatory drugs

A few immunostimulatory drugs (*Endogenous immunostimulants* or *Synthetic immunostimulants*) have been developed to induce humoral or cellular immune responses or both of them against bacterial or viral infections,

immunodeficiency diseases, and cancer. They were classified as follows:

a) **Levamisole** (Ergamisol): Levamisole is a synthetic drug inducing B and T lymphocytes, monocytes, and macrophages. It was used in adjuvant therapy with 5-fluorouracil after surgical resection in patients with Duke's stage C colon cancer. Its disadvantages are allergy, nausea, flu, and muscle pain. Levamisole has been successfully used in combination with polymers for treatment of dermatologic disorders. For example, it was combined with cimetidine for treating recalcitrant warts, and with prednisolone for treating aphthous ulcers of the mouth[21,22].

b) **Thalidomide**: Thalidomide or Immunoprin ($C_{13}H_{10}N_2O_4$) is an immunomodulatory drug. Thalidomide could decrease circulating TNF-α in patients with erythema nodosum leprosum. In contrast, it increased TNF-α in HIV-seropositive patients. Furthermore, its therapeutic effects were determined in severe rheumatoid arthritis and angiogenesis[21].

c) **Isoprinosine** (Inosiplex/ Imunovir): Isoprinosine ($C_{52}H_{78}N_{10}O_{17}$) is a combination of inosine, acetamidobenzoic acid, and dimethylaminoisopropanol. Isoprinosine could enhance the levels of cytokines including IL-1, IL-2, and IFN-γ. It increased the proliferation of lymphocytes against mitogenic or antigenic stimuli. Moreover, Isoprinosine augmented active T-cells and induced T-cell surface markers on prothymocytes. It was used to treat Herpes simplex infections, Epstein-Barr, and Measles viruses. Its disadvantages are minor CNS depressant, transient nausea, and increased level of uric acid in serum and urine[21].

d) **Immunocynin**: Immunocynin is a stable form of haemocynin, a copper-containing protein, found in molluses and arthropods. It was used to treat urinary bladder cancer with poor side effects such as rare-mild fever[21].

e) **Bestatin**: Bestatin, a dipeptide [(2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine, is an immunostimulant with low toxicity which binds to the cell surface of lymphocytes and macrophages and enhances both humoral and cellular immune responses. It is a leucine aminopeptidase and aminopeptidase-B inhibitor. Bestatin possesses antitumor activity and also increase the antitumor activity of bleomycin and adriamycin. Bestatin efficiently prevented the metastasis of P388 leukemia when the antibiotic was constantly injected after tumor inoculation[23]. The dipeptide was immunorestorator in the elderly and cancer patients and HIV-infected subjects. It stimulated granulocytopoiesis and thrombocytopoiesis *in vitro* and could restore them in myelo-hypoplastic man[24].

Bacterial products

The immunostimulatory effects of bacteria and bacterial products are due to the release of cytokines. Live bacillus Calmette-Guerin (BCG) is an attenuated, live culture of the bacillus of Calmette and Guerin strain of *Mycobacterium bovis.* Its mechanism of action includes: a) induction of a granulomatous reaction at the site of administration, and b) prevention and treatment of carcinoma types. Furthermore, BCG enhances both B and T cell-mediated responses leading to phagocytosis and resistance to infection. Its disadvantages are hypersensitivity, fever, shock, and immune complex disease[21].

Recombinant cytokines

Several interferons and interleukins are suggested to stimulate effective immune responses. Interferons could be obtained from trout leucocytes after stimulation with mitogens. It was able to cause an *in vitro* resistance against pancreatic necrosis virus in trout cells. In mammalian, low doses of interferon could induce stable positive results without side effects. On the other hand, vaccination of animals with the recombinant IL-2 against different infections increased the protective effects. However, IL-2 was a very toxic compound in high doses causing side effects such as fever and diarrhea. The purified cytokines showed unsatisfactory results in clinical trials, because the immune responses were produced by a mixture of cytokines generated by the immune cells, but not against a single cytokine. Thus, the enhancers of nonspecific cytokine synthesis may improve immune responses and solve this problem[4]. Thus, recombinant cytokines are produced recently in different expression systems (*e.g*., plants) and used in clinical trials such as interferons, TNF-α and IL-2[25]. ***Complex carbohydrates***

Several types of the complex carbohydrates were described as follows:

a) **Glucans**: An important class of immunostimulants is the β-(1→3)-linked chain of glucose units. The main chain has β-(1→6)-branched glucose units. The β-glucans were obtained from highly conserved structural components of cell walls in fungi, algae, yeast, and have a broad range of molecular weights from 5 to 200 kDa. The length and frequency of these branches vary depending on different sources. β-glucan was used to stimulate anti-tumor mechanisms (*e.g*., increased macrophage activity) and to enhance host resistance to a variety of microbial pathogens in mammalian. Glucan might also be helpful to prevent the carcinogenic effects of aflatoxin. β-glucan was consideredas a stimulator of cellular immunity. Indeed, mammalian macrophages or monocytes have specific receptors for *β*- glucans and produce mediators such as cytokines (*e.g*., IL-1, IL-9, TNF-α) and prostaglandins in the presence of glucans[26, 27]. In Japan, the β-glucans such as Lentinan derived from the Shiitake mushroom and Polysaccharide-K derived from *Coriolus versicolor* were licensed as anti-cancer drugs[28]. Lentinan could induce protective Th1 immune responses to control the proliferation of malaria parasites red blood cells by stimulating maturation of DCs, increasing the expression of MHCII, CD80/CD86, Toll-like receptors (TLR2/TLR4) and the level of IL-12, and preventing the adverse effects of Tregs[29,30]. The main roles of glucans were detected in cancer treatment, infection immunity, stress reduction, and restoration of damaged bone marrow. A mixture of polysaccharides isolated from the cell walls of *Saccharomyces cerevisiae* named as zymosan could potently stimulate macrophages and induce the release of cytokines from neutrophils. Indeed, β-glucan in zymosan was identified as its effective component for non-specific immunomodulation. In addition, β-glucan could reverse myelosuppression generated by chemotherapeutic drugs via targeting the C3 fragment of complement and circulating antibodies. The recent studies have shown that daily therapy with soluble or insoluble β-glucan led to a 70%-95% reduction in tumor size. Indeed, after the binding of antibodies on the surface of cancer cells, C3 fragments of complement could coat the cancer cells. Then, β-glucan-primed cells, such as blood neutrophils, macrophages, and NK cells specifically recognized these complement-antibody complexes and killed the tumor cells. In fact, the cooperation of β-glucan with anti-tumor antibodies is an effective approach in combination therapy[28].

b) **Trehalose**: Trehalose dimycolate (TDM), Muramyl dipeptide (MDP), and Lipopolysaccharides (LPS) as the bacterial products promote the production of antibody, stimulate activation of lymphocytes, and elicit specific immunity against different bacterial infections. Trehalose dimycolate, a glycolipid present in the cell wall of Mycobacteria is a potent immunostimulant that limits tumor growth and enhances resistance against bacterial, parasitic, and viral infections. It can interact with membranes due to its amphipathic properties. TDM primes murine macrophages to

generate nitric oxide (NO) and to develop anti-tumoral activity. As an adjuvant, TDM enhances both cellular and humoral immunity, but elicits a stronger cellular response. TDM could induce potent immune responses against malaria antigens in mice as compared to groups immunized with malarial antigens and Freund's adjuvant. The reports showed that the protective effect of TDM is reduced in macrophage-depleted mice injected with silica particles indicating the role of macrophages. T lymphocytes were not necessary for TDM to prime peritoneal macrophages. Trehalose diesters could induce IL-12p40 and IFN-γ mRNA[31,32].

c) **Prebiotics**: Prebiotics are indigestible fibers that increase beneficial gut commensal bacteria resulting in improvement of the host's health. Prebiotics, such as fructooligosaccharide, mannanoligosaccharide, inulin, or β-glucan, are called immunosaccharides. They directly enhance innate immune responses including phagocytic activation, neutrophil activation, activation of the alternative complement system, and increased lysozyme activity. Immunosaccharides directly activate the innate immune system by interacting with pattern recognition receptors (PRR) expressed on innate immune cells. They can also associate with microbe associated molecular patterns (MAMPs) to activate innate immune cells. Indeed, probiotics activate the innate immune system in two ways: a) by directly stimulating the innate immune system and b) by enhancing the growth of commensal microbiota[33].

Immunostimulants used in vaccines

Vaccines contain a wide range of immunostimulants. For example, an adjuvant heat-labile enterotoxin from *Escherichia coli* (LT), administered as an immunostimulant (LT-IS) patch on the skin may further enhance immune responses to influenza vaccine in the elderly[34]. Also, the immune activation mediated by LT-IS improved the potency of generating Alzheimer's disease (AD)-specific vaccination responses as an adjuvant in the clinical trial[35]. Co-administration of a potent adjuvant in IS patches containing heat-labile enterotoxin from *E. coli* placed on the skin at the site of DNA vaccination significantly increased anti-influenza antibody immune response[36]. Adjuvants enhance and modulate immune responses to antigens. This is important when the purified antigens do not elicit the effective innate or adaptive immune systems. Adjuvants are different in the types and levels of immune responses. Expected advantages of adjuvants contain stronger immune priming, effective immune responses in low-response populations (*e.g.*, the elderly or immuno-compromised patients), the use of smaller amounts of the antigen, and safety profile[37]. New adjuvants have already applied to more efficient influenza vaccines, as well as vaccines targeting hepatitis B (HBV) and human papillomavirus (HPV) [38]. On the other hand, CpG oligonucleotides and imiquimod drugs (an antiviral agent) could activate dendritic cells, induce *in situ* maturation and migration of DCs, and augmented both humoral and cellular immune responses[39]. The unmethylated CpG motif in bacterial DNA was identified as a B-cell stimulating adjuvant, and synthetic oligodeoxynucleotides (ODNs) containing the CpG motifs were shown to induce potent therapeutic activities in different infections and tumor animal models. Imiquimod was topically used for patients with anogenital warts as well as basal-cell carcinoma. The studies indicated that CpG ODNs and imiquimod (resiquimod) drugs act as synthetic ligands for TLR9 and TLR7, respectively, and both stimulate efficiently DC maturation[39].

Plant-derived immunostimulants

Natural plant product promote various activities such as anti-stress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and antimicrobial properties, due to the active substances such as alkaloids, flavanoids pigments, phenolics, terpenoids, steroids, and essential oils. Medicinal plants have been known as immunostimulants, growth promoters, immune enhancers, where they act as antibacterial and antiviral agents to the host immune system. Unfortunately, the mechanisms were not understood[40]. Some medicinal plants were described as following:

a) ***Ocimum sanctum*** (Tulsi): Leaves of *O. sanctum* containing water-soluble phenolic compounds and various other constituents may act as an immunostimulant. Leaves extract of *O. sanctum* affected both specific and nonspecific immune responses. It stimulated both antibody response and neutrophil activity[5,41].

b) ***Phyllanthus emblica*** (Amla): *P. emblica* has antioxidant, anti-fungal, anti-microbial, and anti- inflammatory activities. Amla fruit pulp contains a large amount of vitamin C as an immunostimulant[5,41].

c) ***Azadirachta indica*** (Neem): *A. indica* possesses antihuman immunodeficiency virus, anti-tumor, and antimicrobial activities. Azadirachtin, a triterpenoid derived from *A. indica*, enhanced respiratory burst activities, the leukocyte count and the primary and secondary antibody responses against SRBC (sheep erythrocytes) in tilapia[5,41].

d) ***Solanum trilobatum*** (Purple Fruited Pea Eggplant): The herbal extract of *S. trilobatum* possesses a broad spectrum of antibiotic, antibacterial and anticancer activities. A study showed that the water-soluble fraction of *S. trilobatum* significantly enhanced the production of reactive oxygen and decreased the percentage of mortality following a challenge with *Aeromonas hydrophila*[5,41].

e) ***Eclipta alba*** (Bhringraj): *E. alba* possesses several medicinal properties. The methanol extracts of *E. alba* significantly increased the phagocytic index, antibody titer and WBC count in mice[5,41].

f) ***Zingiber officinale*** (Ginger): The extracts of *Z. officinale* contain polyphenol compounds which have a high antioxidant activity. Moreover, it showed a significant increase in proliferation of neutrophils, macrophages, and lymphocytes, as well as it enhanced phagocytic, respiratory burst, lysozyme, bactericidal and antiprotease activities[5,41].

g) ***Echinacea*** (purple coneflowers) and ***Allium sativum*** (garlic): *Echinacea* and *A. sativum* improved the gain in body weight, survival rate and resistance against challenge infection of *Aeromonas hydrophila*. Both compounds developed resistance to cold stress during the winter season[5,41].

h) ***Camellia sinensis*** (Green tea): Green tea extracts possess biological activity including antioxidant, antiangiogenesis, and anti-proliferative activities that are related to the prevention and treatment of various forms of cancer[5,41].

i) ***Aloe vera****:* Oral administration of *A. vera* could enhance the specific and non-specific immune responses and increase lysozyme activity, serum bactericidal potency, and the total protein and IgM levels[5,41].

j) ***Cynodon dactylon*** (Bermuda Grass): The antiviral activity of *C. dactylon* was confirmed to prevent white spot syndrome virus (WSSV) infection with no mortality and no signs of WSD (White spot disease) [5,41].

k) ***Achyranthes aspera*** (Prickly Chaff Flower): *A. aspera* showed both specific and non-specific immunity revealed by higher levels of serum antibody and also serum antiproteases in fish. Moreover, the level of serum globulin and RNA/DNA ratio of the spleen were also significantly enhanced in the fish fed with *A. aspera*[5,41].

m) ***Nyctanthes arbortristis*** (Night-flowering Jasmine): *N. arbortristis* possesses hepatoprotective, anti-leishmanial, antiviral and antifungal activities. The extract of *N. arbortristis* significantly enhanced serum lysozyme, complement activities and cellular reactive oxygen species (ROS), reactive nitrogen intermediate (RNI) and myeloperoxidase (MPO) production[5,41].

n) **Fermented vegetable product** (FVP): The phagocytic activities, the activity of lysozyme, and superoxide generation of peritoneal leukocytes enhanced in fish fed with the FVP supplemented diet[5,41].

o) **Saffron***:* Saffron, a spice derived from the flower of *Crocus sativus*, is rich in carotenoids. Carotenoids are lipophilic molecules accumulating in lipophilic compartments including lipoproteins and/or membranes. Two main natural carotenoids of saffron, crocin, and crocetin, are responsible for its color[42]. Saffron and its components were suggested as promising candidates for cancer prevention[43]. The mechanisms underlying cancer chemopreventive activities of carotenoids contain modulation of carcinogen metabolism, regulation of cell growth and cell cycle progression, inhibition of cell proliferation, antioxidant activity, immune modulation, enhancement of cell differentiation, stimulation of cell-tocell gap junction communication, apoptosis and retinoid dependent signaling. The immunomodulatory activity of saffron was determined on driving toward Th1 and Th2 limbs of the immune system[42]. Carotenoids increase the proliferative response of T and B lymphocytes to mitogens, the activity of natural killer cells, the number and activity of cytotoxic T-cells, macrophage tumor-killing activity and also induce the secretion of TNF-α in an animal model. These effects are involved in preventing tumor growth, killing tumors and lowering tumor burden. Different carotenoids were used as main phytonutrients to inhibit the development of tumors *in vitro* and *in vivo*[44]. For example, a single treatment with crocin significantly decreased tumor size in a mouse model[43].

Animal originated immunostimulants

There are some immunostimulants derived from animals. For example, chitin and chitosan are the non-specific immunostimulators which are protective against infections for a short time. Also, fermented products of chicken egg (EF203) containing immunoactive peptides showed immunomodulatory effects when administered orally to rainbow trout, *Oncorhynchus mykiss*. Fish treated with EF203 displayed an increased resistance to both natural and experimental β-haemolytic streptococcal infection[41]. Moreover, chitosan, the deacetylated derivative of chitin, has shown strong anti-microbial activity depending on its degree of deacetylation and molecular weight. Both oligomers of chitin and chitosan were effective in enhancing the migratory activity of macrophages. Furthermore, chitosan could activate the production of cytokines such as IL-1β, TNF-α, and reactive oxygen intermediates to promote the defense system against microbial infections[45]. On the other hand, glycated chitosan (GC) as an immunoadjuvant was used in combination with phototherapy for cancer treatment in animal models. *In vitro* studies also showed that after incubation of GC with macrophages, it could significantly stimulate the secretion of TNF-α[46].

Conclusion

Immunomodulators are divided into several groups including physiological products, cytokines, host defense peptides, microbial products, probiotics, synthetic chemical compounds, herbal products, adjuvants, and polysaccharides. Immunostimulants represent a promising class of drugs for the treatment of infectious disorders and cancer. Herbal extracts and animal originated product have a potential application as an immunostimulant, because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. Most of the herbs and herbal extracts can be given orally, which is the most convenient method of immunostimulation in a dose dependent approach. Recently, carbohydrate-based immunostimulants that target Toll-like receptor 4 (TLR-4) and cluster of differentiation 1D (CD1d) receptors as vaccine adjuvants are underway. Also, the incorporation of immunostimulants into nanomaterials has shown a novel approach to enhance the immunostimulation properties. However, further studies are needed to identify effective immunostimulants without adverse side effects and determination of their mechanism of action.

References

1. Chaplin DD. Overview of the Immune Response. J Allergy Clin Immunol. 2015; 125:1-41.

2. Meagher MW. Immune system structure and function. Encyclopedia of Health Psychology. 2014; 1-9.

3. Carter D, Reed SG. Role of adjuvants in modeling the immune response. Curr Opin HIV AIDS. 2010; 5 (5):409-13. https://doi.org/10.1097/COH.0b013e32833d2cdb

4. Galeotti M. Some aspects of the application of immunostimulants and a critical review of methods for their evaluation. J Appl Ichthyol. 1998; 14 (3-4): 189-99. https://doi.org/10.1111/j.1439-0426.1998.tb00641.x

5. Dhama K, Saminathan M, Jacob SS, Singh M, Karthik K, Amarpal, Tiwari R, Sunkara LT, Malik YS, Singh RK. Effect of immunomodulation and immunomodulatory agents on health with some bioactive principles, modes of action and potent biomedical applications. Int J Pharmacol. 2015; 11 (4):253-90. https://doi.org/10.3923/ijp.2015.253.290

6. Schultz KT, Grieder F. Structure and Function of the Immune System. Toxicol Pathol. 1987; 15(3):262-264. https://doi.org/10.1177/019262338701500301

7. Rajasekaran A, Venkatasubramanian G, Berk M, Debnath M. Mitochondrial dysfunction in schizophrenia: Pathways, mechanisms and implications. Neurosci Biobehav Rev. 2015; 48:10-21. https://doi.org/10.1016/j.neubiorev.2014.11.005

8. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma Clin Immunol. 2018; 14:49-59. https://doi.org/10.1186/s13223-018-0278-1

9. Cruvinel WDM, Júnior DM, Antônio J, Araújo P, Tieko T, Catelan T. Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. Bras J Rheumatol. 2010; 50(4):434-461. https://doi.org/10.1590/S0482-50042010000400008

10. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma Clin Immunol. 2011; 7:1-8. https://doi.org/10.1186/1710-1492-7-S1-S1

11. Nielsen HG. Exercise and Immunity. In: Current Issues in Sports and Exercise Medicine. 2016. p. 121-42. Available from: https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

12. Advances in Exercise Immunology - UQ eSpace. Available from: https://espace.library.uq.edu.au/view/UQ:145869.

13. Turvey SE, Broide DH. Innate Immunity. J Allergy Clin Immunol. 2010; 125:24-32. https://doi.org/10.1016/j.jaci.2009.07.016

14. Sattler S. The Role of the Immune System beyond the Fight against Infection. 1003rd ed. London: Springer International Publishing; 2017. p. 3-14. https://doi.org/10.1007/978-3-319-57613-8_1

15. John H Humphrey SSP. Evolution of the immune system. Encycl Br. 2020; 1-3.

16. Travis J. On the Origin of the Immune System. Science. 2009; 324(5927):580-582. https://doi.org/10.1126/science.324_580

17. Panda S, Ding JL, Alerts E. Natural Antibodies Bridge Innate and Adaptive Immunity. J Immunol. 2015; 194:13-20. https://doi.org/10.4049/jimmunol.1400844

18. Janeway CA JR, Travers P WM. Immuno biology: The Immune System in Health and Disease. 5th ed. New York: Garland Science; 2001. 1-884 pp.

19. Petrunov B, Nenkov P, Shekerdjiisky R. The role of immunostimulants in immunotherapy and immunoprophylaxis. Biotechnol & Biotechnol Eq. 2007; 21(4):454-63. https://doi.org/10.1080/13102818.2007.10817494

20. Labh SN, Shakya SR. Application of immunostimulants as an alternative to vaccines for health management in aquaculture. Int J Fish Aquat St. 2014; 2 (1):153-6.

21. Patil US, Jaydeokar AV, Bandawane DD. Immunomodulators: A pharmacological review. Int J Pharm Pharm Sci. 2012; 4 (1):30-6.

22. Biswajit D, Suvakanta D, Chandra CR, Jashabir C. An overview of levamisole hydrochloride with immunostimulant activity. Am J Pharm Health Res. 2014; 2(4):1-9.

23. Jain M, Jain A, Khare B, Jain DK, Khan R, Jain D. An Update on the Recent Emergence of Candida auris. Asian Journal of Dental and Health Sciences. 2022; 2(1):14-9. https://doi.org/10.22270/ajdhs.v2i1.11

24. Mathe G. Bestatin, an aminopeptidase inhibitor with a multipharmacological function. Biomed Pharmacoter. 1991; 45:49-54. https://doi.org/10.1016/0753-3322(91)90122-A

25. Sirko A, Vanek T, Gora-Sochacka A, Redkiewicz P. Recombinant cytokines from plants. Int J Mol Sci. 2011; 12 (6):3536-52. https://doi.org/10.3390/ijms12063536

26. Sahoo PK, Mukherjee SC. Effect of dietary β-1, 3 glucan on immune responses and disease resistance of healthy and aflatoxin B1-induced immunocompromised rohu (Labeo rohita Hamilton). Fish Shellfish Immunol. 2001; 11 (8):683-95. https://doi.org/10.1006/fsim.2001.0345

27. Madrigal-Bujaidar E, Morales-González JA, Sánchez- Gutiérrez M, Izquierdo-Vega JA, Reyes-Arellano A, Álvarez- González I, Pérez-Pasten R, Madrigal-Santillán E. Prevention of aflatoxin B1-induced DNA breaks by β-D-glucan. Toxins (Basel). 2015; 7 (6):2145-58. https://doi.org/10.3390/toxins7062145

28. Vetvicka V. Glucan-immunostimulant, adjuvant, potential drug. World J Clin Oncol. 2011; 2 (2):115-9. https://doi.org/10.5306/wjco.v2.i2.115

29. Zhou LD, Zhang QH, Zhang Y, Liu J, Cao YM. The shiitake mushroom-derived immuno-stimulant lentinan protects against murine malaria blood-stage infection by evoking adaptive immune-responses. Int Immunopharmacol. 2009; 9(4):455-62. https://doi.org/10.1016/j.intimp.2009.01.010

30. Sajeevan TP, Philip R, Bright Singh IS. Dose/frequency: A critical factor in the administration of glucan as immunostimulant to Indian white shrimp Fenneropenaeus indicus. Aquaculture. 2009; 287(3):248-52. https://doi.org/10.1016/j.aquaculture.2008.10.045

31. Parant M, Audibert F, Parant F, Chedid L, Soler E, Polonsky J, Lederer E. Non-specific immunostimulant activities of synthetic Trehalose-6, 6'-Diesters (Lower Homologs of Cord Factor). Infect Immun. 1978; 20 (1):12-9. https://doi.org/10.1128/iai.20.1.12-19.1978

32. Oswald IP, Dozois CM, Petit JF, Lemaire G. Interleukin-12 synthesis is a required step in Trehalose Dimycolate-induced activation of mouse peritoneal macrophages. Infect Immun. 1997; 65 (4):1364-9. https://doi.org/10.1128/iai.65.4.1364-1369.1997

33. Jat D, Thakur N, Jain DK, Prasad S, Yadav R. Iris ensata Thunb: Review on Its Chemistry, Morphology, Ethno Medical Uses, Phytochemistry and Pharmacological Activities. Asian Journal of Dental and Health Sciences. 2022; 2(1):1-6. https://doi.org/10.22270/ajdhs.v2i1.9

34. Frech SA, Kenney RT, Spyr CA, Lazar H, Viret JF, Herzog C, Glück R, Glenn GM. Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch. Vaccine. 2005; 23 (7):946-50. https://doi.org/10.1016/j.vaccine.2004.06.036

35. Davtyan H, Ghochikyan A, Hovakimyan A, Petrushina I, Yu J, Flyer D, Madsen PJ, Pedersen LO, Cribbs DH, Agadjanyan MG. Immunostimulant patches containing Escherichia coli LT enhance immune responses to DNA- and recombinant proteinbased Alzheimer's disease vaccines. J Neuroimmunology. 2014; 268 (1-2):50-7. https://doi.org/10.1016/j.jneuroim.2014.01.002

36. Mkrtichyan M, Ghochikyan A, Movsesyan N, Karapetyan A, Begoyan G, Yu J, Glenn GM, Ross TM, Agadjanyan MG, Cribbs DH. Immunostimulant adjuvant patch enhances humoral and cellular immune responses to DNA immunization. DNA Cell Biol. 2008; 27 (1): 19-24. https://doi.org/10.1089/dna.2007.0639

37. Garçon N, Leroux-Roels G, Cheng WF. Understanding modern vaccines: Perspectives in Vaccinology. Elsevier BV. 2011; 1: 89-113. https://doi.org/10.1016/j.pervac.2011.05.004

38. Pasquale AD, Preiss S, Da Silva FT, Garçon N. Vaccine adjuvants: from 1920 to 2015 and Beyond. Vaccines (Basel). 2015; 3 (2):320-43. https://doi.org/10.3390/vaccines3020320

39. Mizumoto N, Gao J, Matsushima H, Ogawa Y, Tanaka H, Takashima A. Discovery of novel immunostimulants by dendritic-cell based functional screening. Blood. 2005; 106 (9):3082-9. https://doi.org/10.1182/blood-2005-03-1161

40. Khatri S, Jain DK. Autism spectrum disorder (ASD): past, present and future. CIBTech Journal of Pharmaceutical Sciences. 2018; 7(4):1-25.

41. Bairwa MK, Jakhar JK, Satyanarayana Y, Reddy AD. Animal and plant originated immunostimulants used in aquaculture. J Nat Prod Plant Resour. 2012; 2(3):397-400.

42. Bolhassani A, Khavari A, Bathaie SZ. Saffron and natural carotenoids: Biochemical activities and anti-tumor effects. Biochim Biophys Acta. 2014; 1845:20-30. https://doi.org/10.1016/j.bbcan.2013.11.001

43. Khavari A, Bolhassani A, Alizadeh F. Chemoimmunotherapy using saffron and its ingredients followed byE7-NT (gp96) DNA vaccine generates different anti-tumor effects against tumors expressing the E7 protein of human papillomavirus. Arch Virol. 2015; 160(2): 499-508. https://doi.org/10.1007/s00705-014-2250-9

44. Bolhassani A. Cancer chemoprevention by natural carotenoids as an efficient strategy. Anticancer Agents Med Chem. 2015; 15(8):1026-31. https://doi.org/10.2174/1871520615666150302125707

45. Mastan SA. Use of immunostimulants in aquaculture disease management. Int J Fish Aquat St. 2015; 2(4):277-80.

46. Chen WR, Sarker A, Liu H, Naylor MF, Nordquist RE. Effects of immunostimulants in phototherapy for cancer treatment. Biophotonics and Immune Responses IV. 2009; 71780A. https://doi.org/10.1117/12.808019