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Evaluation of Immunomodulatory Effect: Selection of the Correct Targets for Immunostimulation Study

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Abstract: Problem statement: Numerous plants or remedies that are traditionally used for various diseases had been claimed to maintain general good health, particularly the immune system. With the advanced understanding on immunology and ethnopharmacology, study on the interaction of this herb with the immune system is critical to understand the safety and its efficacy as a potent immunomodulatory agent. **Approach:** Selecting proper immune cells from a suitable immune organ allowed for the understanding on the mode of immunomodulation. Lymphocytes isolated from mammalian thymus, spleen and bone marrow represented great candidates for this immunomodulatory study. **Results:** A number of herbal extracts including that of Rhaphidophora korthalsii and compounds isolated from this plant, namely lectin and zerumbone, had been identified as potent immunostimulators. **Conclusion/Recommendations:** Future studies are needed to elucidate the mechanism underlaying activites of these immunomodulatory agents.

Key words: Immunomodulation, PBMC, spleen, mitogen

INTRODUCTION

Immunomodulator is the substances that are capable of interacting with the immune system to up-regulate or down-regulate specific aspect of the host response (Stanilove *et al*., 2005; Utoh-Nedosa *et al*., 2009). It is also known as biologic response modifier or immunoregulator which is function as drug leading predominantly to a non-specific stimulation of immunological defense mechanisms (Tzianabos, 2000). Immunomodulators may include some bacterial product, lymphokines and plant derived substances. The effects of immunomodulator can be classified into three which are stimulation, suppression and restoration of the immune system. Unlike vaccine, most of immunomodulator agents are not real antigens but antigenomimetics or so called mitogens. Due to their actions as a non-specific and non-antigens properties, they do not stimulate the development of memory lymphocytes. Thus the effect of immunomodulator agents towards specific immune system will be reduced after a short of period of time (Wagner, 1999).

Immunomodulators are used in clinical practice to stimulate and normalize an immune system activity. Most of the immunomodulator agents play their role in maintaining the immune system by increasing T cell immunity, decreasing or blocking the suppressor activity, stimulating the Natural Killer cells (NK cells) and interferon production as well as inducing specific cytokine production by activated target cells (Gabius, 2003; Stanilove *et al*., 2005; Lam *et al*., 2010). Nowadays, the application of imunomodulators is the practice of modern method for the correction of immunodeficiencies. According to Ganju *et al*. (2003), immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases particularly when host defense mechanism has to be activated under the conditions of

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impaired immune response or when a selective immunosuppression is desired in situation like autoimmune disorders. The immuno-corrective properties of immunomodulators also can be successfully applied in the treatment of oncological diseases.

According to Wagner (1999), immunomodulators might be effective for the prophylaxis of metastases after removal of the primary tumor. It is also essential to facilitate the targeting and the recognition of diseased cells by immuno-competent cells. The immunocompetent cells play a principle role in the processes of eliminating tumors cells. These cells can be directed specifically towards the selected type of tumor cell or used in order to improve the immune resistance of the whole organism. Recently, the fundamental field of immunomodulator has involved in many medical areas such as treatment of organ rejection after transplantation, recovery from infectious diseases, primary immunodeficiencies and to stabilize the immune system of HIV positive patients.

Due to broad application of its action, immunomodulators are becoming very popular in the worldwide natural health industry as people start to realize the importance of a healthy immune system. However, selection of a proper target cells and markers are utmost important to evaluate the immunostimulatory effect of that particular substance.

Immune system: The immune system is the crucial body system which helps to protect the body against a wide variety of pathogens (Froy *et al*., 2007). Each structure of the immune system has a relatively fixed architecture of specialized organs, compound of lymphoid tissues, cells and chemicals. It has the ability to respond to antigen such as microbe or various macromolecules that is recognized as non-self or antigen. The success of this system in defending the body relies on an incredibly elaborate and dynamic regulatory communication networks, that involves multiple and functionally differing cell types which provide a large variety of defend mechanisms. The outcome is a sensitive system of check and balances that produces an immune response that is prompt, appropriate, effective and self-limiting (Becker, 2006; Zane, 2001).

Type of immune system: The human immune system has two defend system which can be divided into innate (or natural) immunity and adaptive (or acquired) immunity (Aagaard-Tillery *et al*., 2006). Natural or innate immunity is the body's first defense mechanisms against a foreign antigen. This mechanism do not required specific recognition of an antigen by the immune system (Vollmar, 2005). However, in situation in which the antigen escapes this natural protective mechanism and invades the host, another set of antigen specific and powerful defense mechanism are triggered due to "memory" of the cells. These mechanisms are known as adaptive (or acquired) immunity (Becker, 2006; Zane, 2001).

The acquired immunity can be subdivided into humoral immunity and cell mediated immunity which involved the reaction of lymphocytes. In the humoral immunity, it involves the secretion of antibodies which is B-lymphocytes that bind the antigen or enhances phagocytosis through opsonization to remove the stimulating antigen. Thus, it main mechanism in the body is to remove and neutralize the toxic. In contrast, the cell mediated immunity is mediated by the cytolytic T lymphocyte which can specifically recognize and activate the macrophages or kill the infected cells directly (Parslow, 2001). Both B and T lymphocytes are responsible in defending the immune system against infectious pathogen.

Organ and cells of the immune system: The immune system consists of cells and their secretory products, various lymphoid tissues and organ where these components are recognized and localized (Zane, 2001). The main components of the immune system are lymphoid tissue which divided into primary and secondary lymphoid tissue, cellular component such as lymphocytes and soluble components (mediator) like cytokines, antibodies and complement component. Two organs are designated as primary lymphoid tissues which are bone marrow and thymus whereas lymph nodes, spleen and scattered lymphoid tissue are designated as secondary lymphoid tissue. Thymus and spleen are group of primary and secondary lymphoid tissue, respectively whereas PBMC is a cellular component in the immune system which play an important role in the immune system and serve as a reservoir for foreign antigens.

Peripheral blood mononuclear cells: The human body is nourished by a dynamic circulatory system composed of cellular components of which have a relatively rapid turnover rate (Vlata *et al*., 2006). PBMC are classified as a fluid connective tissue, which can be termed as cells suspended in a fluid matrix functioning to connect the entire biological system at the physiological level. Blood cells also involve in the first line of the immune defense system, using an arsenal of neutrophils, eosinophils, basophils, B cells, T cells and monocytes to defend against foreign substances, injury and provide a protective barrier between the external and internal (Liew *et al*., 2006). These peripheral blood

mononuclear cells play crucial role in the immune defense during the pathological conditions by stimulating the process of activation, cell division and differentiation to generate a large pool of activated effector T-cells which react to the antigen (Khanduja *et al*., 2006; Winkler *et al*., 2005).

Thymus: Thymus is a primary lymphoid tissue known as a dedicated organ for T cell development (Wu, 2006). The thymus gland is a bilobeb structure, located in the thorax. Each lobe contains lymphoid cells (thymocytes) that form a tightly packed outer cortex and an inner medulla. The cortex contains the immature and proliferating cells, while the medulla contains of the more mature cells, indicating the existence of a maturation gradient from the cortex to medulla (Zane, 2001; Wu, 2006). During development, T cell progenitor originating from bone marrow migrates into the thymic epithelium. These progenitors are released in waves from bone marrow into the blood stream and then imported periodically into the thymus. The thymus provides a unique microenvironment where thymocytes proliferate and differentiate, passing through series of discrete phenotypic stages that can be identified by distinctive patterns of expression of various cell surface proteins (Godfrey and Zlotnik, 1993). The major function of the thymus is in the maturation and selection of an antigen specific T-lymphocytes from marrow derived precursor cells (Anderson *et al*., 1996). During the maturation process, the T-lymphocytes acquire surface receptors such as T cell receptors (Tcr) which is important in antigen recognition and the T cell activation process as well as in the identification of the cell's phenotype (Alvarez *et al*., 2006; Zane, 2001).

The thymus involutes (diminishes in size) with age, with only medullary remnants remaining. This involution of the thymic lymphoepithelial component is one of the most prominent features of ageing in the immune system. Based on study in animal and human, it is generally accepted that the volume of true thymic tissue attains maximum size at puberty, after that, it decreases gradually (Shanker, 2004). Therefore, with ageing, the thymic tissue weakens as a source of naïve T lymphocytes (Romanyukha and Yashin, 2003). The reduced T cell output, together with an increase in apoptosis of naïve T-cells limits the ability of aged individuals to respond to newly encountered antigens (Leposavic *et al*., 2006). The markedly reduced size of the naïve T-cell subpopulation together with an increased number of memory cells in the periphery, is a clear-cut characteristic of ageing in the immune cells (Romanyukha and Yashin, 2003). However, recently, Mocchegiani *et al*. (2006) reported that certain nutrition might effects thymic physiology. Studies on animals have shown that oral zinc supplement in aged mice induced thymus re-growth couple with an increase in the production of thymic hormone. As a result, this study suggested that dietary zinc supplement during the whole life-span might prevent the thymic involution during ageing processes.

Spleen: The spleen is the largest lymphoid organ in the body (Fedeler and Blatteis, 2006). It is a secondary lymphoid tissue and located in the left upper quadrant of the abdomen. It contains two compartments which are white pulp and red pulp with a marginal zone in between. The red pulp is composed of blood–filled vascular sinusoids while the white pulp is lymphoid tissue consisting mainly of lymphocytes surrounding the arteries. In the marginal zone it composes mainly B cells and macrophages (Parslow, 2001; Abbas *et al*., 2000). In this region also, the bloodstream passes through an open system of reticular cells and fibers in which various myeloid lymphoid cells are located. The T cells in the spleen are located in the periarteriolar lymphoid sheath. Macrophages in the marginal zone are well equipped to recognize pathogen and filter the blood by virtue of unique combination of pattern recognition receptors. They interact with a specific set of B cells that can be found only in a marginal zone and that are able to react rapidly to bacterial antigens in particular. In fact, around half of total body blood volume will pass through the spleen to filter the pathogen by using the sophisticated macrophages filtration system (Engwerda *et al*., 2005; Butcher, 2005).

In addition, spleen is also known to play several functions in the immune system. It plays an important role in defense against blood-borne pathogen because it consists of lymphocytes, dendritic cells, natural killer cells, red blood cells and macrophages. Besides to capturing antigens from the blood that passes through the spleen, migratory macrophages and dendritics cells bring antigens to the spleen via the bloodstream. This event will initiate an immune response by producing large amounts of antibody. Spleen also acts as reservoir area for blood when blood is needed in an emergency such as hemorrhage. In this situation the muscles in the spleen contract, forcing the stored blood out and back into general circulation. Spleen also destroys and worn-out old blood cells as well as it plays an important role in red blood cell production (erythropoiesis) before birth (Parslow, 2001; Abbas *et al*., 2000; Portillo *et al*., 2004; Fedeler and Blatteis, 2006).

The capability of spleen possessing its role in the immune system linked intimately with the diet.

Previously, Jeffery *et al*. (1997) proved that spleen lymphocytes proliferation was enhanced by palmitic acid rich diet which a group of saturated fatty acid found abundantly in palm oil. This result suggested that low fat diet can boost up the immune system. Subsequently, more recently, Field and his groups found that supplementing diet with additional folate significantly improved the distribution of T cells, increased mitogen responses and corrected most of the aberrant cytokine productions in the spleen (Field *et al*., 2006). Therefore, both results suggested that nutrition plays a crucial role in enhancing the immune system particularly in maintaining spleen health.

Lymphocytes: The lymphocytes are a class of leukocytes normally present in blood (Ndejembi *et al*., 2007). Their primary function is to survey the body and recognize any foreign material that may indicate the presence of virus, bacteria, parasites or tumor cells (Ndejembi *et al*., 2007; Victor, 2007). Lymphocytes can be grouped into different classes depending on their functions. The classes of lymphocytes are B-lymphocytes, T-lymphocytes and Natural Killer cells (NK cells). The relative proportion of T and B cells in peripheral blood accounts for about 75 and 10% respectively, while the remaining 15% are NK cells (Cerqueira *et al*., 2004). All of them can be distinguished from one another and from other leukocytes on the basis of surface marker (Zane, 2001).

Classification of lymphocytes on the basis of surface marker makes use of two important classes of the characteristic which include the Cluster Designation (CD) and the nature of antigen recognition receptor expressed (Parslow, 2001). An important differential feature in antigen recognition by these two lymphocyte population is that B cell recognize native antigen configuration and require helper T cell (CD4+) participation in order for immune response to occur, whereas T cell (CD8+ and CD4+) recognized only a "processed" antigen and in the context of self-MHC molecule (Parslow, 2001).

B-lymphocytes or the B cells are derived and developed from adult bone marrow and fetal liver. For adult mammals, the B cells are produce in the bone marrow and circulate in the blood stream in immature form. The selected phenotype markers which help to differentiate B lymphocytes from others are Fc receptors, class II MHC, CD19 and CD 21. B cells are responsible to produce antibodies (or immunoglobulin) which can bind specific with antigen in humoral immunity (Sen, 2006).

In contrast, the precursors of T lymphocytes originate in the bone marrow and mature in thymus. It is responsible for cell mediated immunity and can work with B cell in the humoral immune response. There are two types of T cell: Helper T lymphocyte (with CD3+, CD4+ and CD8- marker on the cell surface) and Cytolytic T lymphocytes (with CD3+, CD4- and CD8+ marker on the cell surface) and each of them carry different functions. The helper T cells are responsible for macrophage activation and stimulation of B cell growth and differentiation while Cytolytic T lymphocytes are responsible for killing of virus-infected cells, tumor cells and allograft rejection after transplantation. Both the mature B and T lymphocytes will enter into the peripheral lymphoid organs such as lymph nodes, spleen, mucosal and cutaneous lymphoid tissues (Abbas *et al*., 2000).

The natural killer cells (NK cells) are a third population of lymphocytes. They express the CD2 marker, the Fc receptor for IgG molecule (CD16), the IL-2 receptor and elaborate Tissue Necrosis Factor (TNF). These cells are neither T nor B lymphocytes because their lack both the immunoglobulin (Ig) receptors normally present on a B lymphocytes surface and the specific T Cell Receptors (TCR) (Vivier, 2006). It functions as the non-specific killer toward the virus-infected cells and tumor cells (Morretta and Morretta, 2004; Eales, 2003).

Plant mitogens: One of the most important sources of immunomodulator which are being explored extensively currently is come from plants derived substances. There are several plants have been recognized to have mitogenic effect on the immune cells. For examples, *Rhaphidophora korthalsii* was found to stimulate immune cell proliferation, cytokines expression and natural killer cell cytotoxicity *in vitro* and *in vivo* (Yeap *et al*., 2007; 2011a; 2011b). On the other, *Elephantopus scaber* and *Vernonia amygdalina* were found to be the major ingredient in a traditional herbal formula, which carries the immunomodulatory effect (Ho *et al*., 2009; Yeap *et al*., 2010; Hertiani *et al*., 2010). A variety of substances have been discovered that bind to the surface of lymphocytes, thus stimulating them to undergo mitosis (Lao *et al*., 2001).

An example of lymphocyte mitogens are lectins. Lectins are glycoproteins or carbohydrate-binding proteins that have the ability to bind specifically, selectively, free or conjugated saccharides in a reversible way by two or more binding sites (Maciel *et al*., 2004). Some lectins induced lymphocytes proliferation or modulated several immune functions by interaction with their carbohydrate recognized receptors. One of the most dramatic effects of interaction of lections with the lymphocytes is their

mitogenecity through triggering of quiescent, non dividing lymphocytes into a state of growth and proliferation (Bains *et al*., 2005). However, not all lymphocytes respond equally to all lectins (Lao *et al*., 2001). More recently, plant lectins are widely used in laboratory trial as stimuli for *in vitro* assessment of immune cells behavior and activity (Stanilove *et al*., 2005). The first plant lectin discovered was PHA, lectin isolated from red kidney bean (*Phaseolus vulgaris*) by Nowell (Bains *et al*., 2005). PHA has been identified to stimulate blastogenesis of T lymphocytes by interaction with CD2 to stimulate the production of IL-2 and IFN-γ. This lectin primarily stimulates T cell proliferation, although it has a slight effect on B cells. The discovery of lectin-mediated mitogenesis led to the detection of many other mitogenic lectins, such as concanavalin A (Con A) and Pokeween Mitogen (PWM). Con A isolated from jack bean (Canavalia ensiformis) was found to bind specifically to alpha-D-glucopyranosides and alpha-D-mannosepyranosides. It has strong mitogenic effects on T cells but not on B cells. The lectin from *phytollaca* Americana, Pokeweed Mitogen (Pwm), has mitogenic activity on both T and B lymphocytes and induces various types of cytokines including type 1 cytokines (IL-2 and IFN-γ), type 2 cytokine (IL-10) and monokines (IL-6 and TNF-α). Nevertheless, the specific receptor that couple with PWM is currently unidentified (Stanilove *et al*., 2005).

Besides lectin, other plant secondary metabolites including zerumbone (isolated from ginger), damnacanthal (isolated from noni) have also been proved to carry immunostimulatory effect via the examination of immunoproliferation, cell cycle alteration and anticancer cytokines (IL-2 and IL-12) expression (Keong *et al*., 2010, Alitheen *et al*., 2010). Upregulation of these cytokines has been reported to be apotential adjuvants in cancer immunotherapy (Capitini *et al*., 2009).

CONCLUSION

Immunomodulator especially immunostimulator carries number of potential benefit in maintaining strong immune system especially for the cancer patient who always associated with poor immunity while undergoing chemotherapy (Abdulamir *et al*., 2008; Wadekar *et al*., 2009). Herbs or plants which have been widely used in ethnopharmacology are the great source of immunomodulator. However, this bioactivity requires scientific proved to confirm the safety and effective dosage of that particular herb. Selecting the appropriate immune cells and specific immune organ as a study target for immunomodulation research allow the understanding of the interaction of that particular substances with the specific immune cells.

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