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Effect of Concanavalin A on IL-2 Expression from Lymph Node Cells

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Abstract: Lymph nodes are organ and widely distributed in different parts of the body where innate immune responses lead to acquired immunity where autoreactive lymphocytes first encounter tissue-specific self-antigens and are either tolerized or activated. Lymph nodes respond well to mitogens (Concanavalin A) and alloantigens and stimulate the T-lymphocyte proliferation and activate the IL-2 surface receptor for expression of IL-2 *in vitro*. The present review aimed to discuss the pattern of expression of IL-2 and the effect of Concanavalin A in lymph nodes.

Key words: Lymph, mitogen, interleukin, lymphocyte, immune system

INTRODUCTION

The lymphatic system is one of the most complex and vital systems inside the body. It is a part of circulatory system and plays an important role in immune system. The primary purpose of which is to isolate and eliminate infections (Ruddle and Akirav, 2009). Lymph nodes are a small ball or an oval-shaped organ of the immune system, widely distributed throughout the body including the armpit and stomach/gut and linked by lymphatic vessels. These consist of multiple lymphoid lobules surrounded by lymph-filled sinuses and enclosed by a capsule and contains large numbers of lymphocytes, macrophages and Antigen Presenting Cells (APCs) (Tilney, 1971; Willard-Mack, 2006).

Lymph nodes contain macrophages and Dendritic Cells (DCs) which express MHC class II molecules on their cell surface and have potential to activate T cell because T cells bind only those APCs having Major Histocompatibility Complex-II (MHC-II) molecules. DCs are essential antigen presenting cells for MHC class II presentation to naive T cell. MHC class II presentations by DCs that migrate from non lymphoid tissue stimulate T cells and play important role in activation of T cells (Itano and Jenkins, 2003) as shown in Fig. 1. T cells are stimulated and to proliferated 1.5-2 days after they encounter their antigens displayed on dendritic cells. The appearance of tangible body macrophages and a starry sky appearance in the paracortex are indication of increased apoptosis and T cell production.

According to the localization, lymph node classified as superficial lymph nodes, situated in the subcutaneous area and near the skeletal muscular masses and as deep lymph nodes, situated inside the thoracic and abdominal cavity or close to the organs (Dunn, 1954). All superficial lymph nodes are bilateral and classified as:

- Deep cervical lymph nodes, often difficult to localize, the more superficial ones are found in the cervical plane, hidden in the connective tissue that encircles the trachea
- Mediastinum or thoracic lymph nodes situated on the posterior face of the two lobes of the intimately connected thymus
- Pyloric or pancreatic lymph nodes near the margin of the pancreas
- Renal lymph nodes situated between the median margin of kidneys, more often at level of the hilum and in correspondence of the abdominal aorta
- Mesenteric lymph node of lengthened shape that lies between the mesentery membranes, close to the ascending portion of the colon
- Lumbar and caudal lymph nodes localized in proximity to the bifurcation of the aorta

Free antigen and antigen-loaded DCs are transported to the lymph nodes from distal tissues through afferent lymph vessels whereas naive lymphocytes enter these organs in High Endothelial Venules (HEVs). If lymphocytes fail to recognize specific antigen within a few hours to days, they return to the circulation through efferent lymph vessels and the thoracic duct. The lymph nodes have a crucial role in collecting the prerequisite information that allows lymphocytes activation in context of appropriate co-stimulation; T cells undergo clonal expansion and acquire effector functions.

Afferent lymph contains antigen-presenting dendritic cells, T cells and a small proportion of B cells. Lymph flow and cell output rates are low. Efferent lymph contains >98% lymphocytes with a higher proportion of B cells and much higher flow and cell output rates than in afferent

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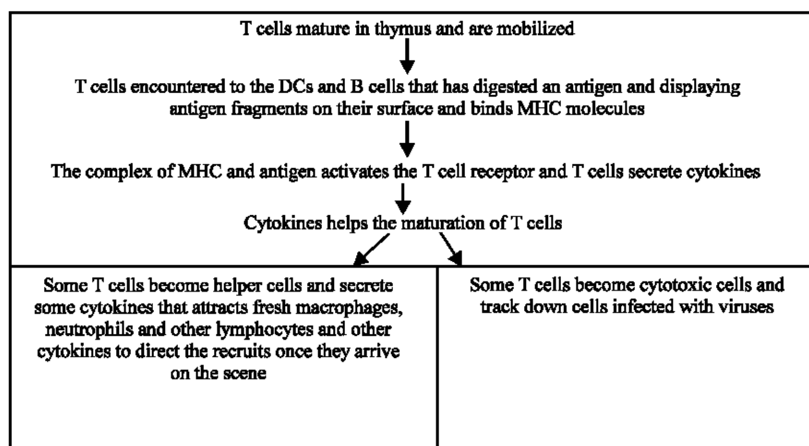

Fig. 1: Flow diagram of activation of T cells (Abbas *et al*., 2007)

lymph. Greater than 90% of efferent lymphocytes are derived from the blood via HEVs. Unlike naive T cells, effector cells can produce cytokines, kill antigen expressing cells (in the case of cytotoxic T lymphocytes) or modify the behaviour of other leukocytes (in the case of T helper cells) and allow them to migrate to the source of their antigen in peripheral tissues. So, effector cells preferentially generate in the skin-draining lymph nodes whereas Mesenteric Lymph Nodes (MLN) or peyer's patches produce mucosa-homing subsets (Von Andrian and Mempel, 2003).

Functions of the lymph nodes in the immune system: They recruit a large numbers of naive lymphocytes from the blood to collect antigen and DCs from peripheral tissues to provide the environment for antigen-specific tolerance or productive primary and secondary effector responses. They also modulate the homing characteristics of effector or memory T cells, targeting them to tissues that contain their cognate antigen and also provide a look-out for central memory cells. The role of lymph nodes in the immune system with particular emphasis on the trafficking signals which makes these organs a crucial interface between the innate and adaptive cellular components of the immune system (Von Andrian and Mempel, 2003). The immunological processes which occur in lymph node takes place in a specialized stromal structure called the reticular meshwork that supports guides and organizes interactions between lymphocytes and APCs (Gretz *et al*., 1996). Particulate antigens are also filtered out of the lymph and destroyed by macrophages. Cytokine expression in lymph node is induced by antigens and principally produces TNF, IL-2 and IFN-γ and other cytokines such as IL-3, IL-4, IL-5, IL-6 and IL-10. IL-2 plays an important aspect in the activation phase of a developing immune response (Litton *et al*., 1994).

LYMPH NODE CULTURE

Cell cultures are derived from dispersed cells taken from original tissue and disaggregated by enzymatic, mechanical or chemical means and provided large numbers of cells suitable for isolation of infected cell and decrease the use of experimental animals. Cell cultures are less expensive and convenient to examine microscopically for evidence of antigenic proliferation and also provided a desirable environment for the detection and identification of many pathogens. Cell culture technology is used for the development of monoclonal antibodies, vaccines and also used for the wide variety of study such as model system, understanding of disease condition (Parkinson disease, diabetes and multiple sclerosis) and examine tissue and organ development (Leland and Ginocchio, 2007). Cells that are cultured directly from organs and tissues are known as primary cell culture. Lymph node culture is a primary cell culture. It is maintenance of growth of cells dissociated from the parental tissue (such as kidney, liver) using the mechanical or enzymatic methods in culture medium using suitable containers under controlled condition (Trowell, 1995). Primary cell culture is of two types depending upon kind of cell in culture:

- Anchorage dependent or adherent cells: cells require attachment for growth are set to be Anchorage dependent cells. For example epithelial tissues, connective tissue (fibroblast), muscle tissue (vascular smooth muscle cells), nervous tissues and many cancerous tumors
- Suspension culture or Anchorage independent cells: cells which do not require attachment for growth or do not attach to the surface of the culture vessels are anchorage independent cells/suspension cells. For example mainly lymphocytes or lymphoblast cells are grown in suspension

There are many methods to produce a single cell suspension of lymph cells from lymph nodes for *in vitro* studies. Single cell suspension from lymph node can be prepared and disrupted mechanically and enzymatically (Koscielny *et al*., 2011) such as collagenase, trypsin or pronase to breakdown the extracellular matrix. Collagenase digestion of pre-labelled lymph nodes has been used for primary lymph node cultures of high endothelial cells from rat lymph node (Ager, 1987). Trypsin and pronase are considered to be protease and to get them detached from the cells culture wall of the dish. Cells are swimming around in the medium and able to transfer in new culture dish with fresh nutrients. Like any other primary culture lymph node culture would retain many of differentiated characteristic of cells *in vivo* thus having the potential for acting as an alternative method to mammalian models. Lymph node can also be cultured *in vitro* by method of organ culture as distinct from tissue culture. Lymph node remain as intact organ, there is no outwandering of cells into the culture medium (Trowell, 1995).

OVERVIEW OF CRYOPRESERVATION

Cryopreservation is a technique which provides optimal conditions for the preservation of mouse lymph node cells in liquid nitrogen using cooling rate techniques and the survival of mouse lymphocytes throughout a procedure for storage at -196°C for the improvement of recovery and the possible extension to the mouse system of cell selection by freezing. After thawing, the survival of cells cools at different rates in cryprotectant such as dimethyl sulphoxide (DMSO, 5 or 10%, v/v) is assessed from the [^{3}H] thymidine incorporation in response to mitogens such as PHA and Con A. The percentage of cryoprotectant in culture is 5-10% and a change in temperature from 1-3°C min^{-1} is believed to produce minimal damage of cells (Thorpe *et al*., 1976). It has been reported that after the transfer of frozen cell to liquid nitrogen after 7 days at -65°C and after 6 months >9000 of the cells were found to be viable and grow normally when injected intraperitoneally into syngeneic mice. The cryopreservation of Con A treated mice lymph node because Con A have ability to caused blastogenic transformation of lymphocyte (Grant, 1976). Freezing of lymphocytes at 1°C min^{-1} by a control procedure has been designed to yield maximum survival. Cooling at 1°C min^{-1} by a controlled, optimized procedure has been shown to yield upto 91% survival of viable peritoneal exudated cells. Cells cooled at either -20 or -75°C min^{-1} showed total inability to respond to stimulation and also showed that the cooling at either 1 or -20°C min^{-1} in the experiment and showed that 72-75% survival of cells frozen and 33% of cell survived when frozen by an alternative procedure (Taylor and Bank, 1988).

The viability of cell in culture medium is also depends upon season. It has been found that activation of both T and B lymphocytes by mitogens is maximal in the Spring and Summer and declined upto 40% of unfrozen control levels in October. Single cell suspensions cooled at 1°C min^{-1} in 10% DMSO. The optimum velocity is determined by using a range of cooling rates and were found that recoveries of viable cells were high from March through July and then declined to minimum levels in January and February (Brock, 1987). Spleen and lymph node lymphocytes of rat have been frozen with DMSO at 1°C min^{-1} and stored at -196°C for 10 min and the functional recovery of the cell populations was monitored by the mitogenic response in culture after thawing. With 5-10% of DMSO in the freezing medium, frozen-thawed lymph node cells were found to retain about 40% of their response to mitogen and the concentrations of DMSO induced >10 fold higher than that present in the culture medium after freezing and thawing and the removal of adherent cells from the spleen cell population and also produced an augmentation response to mitogen but have an inhibitory effect on suppressor cell functions present in spleen cell populations (Hem, 1976).

APPLICATIONS OF CRYOPRESERVATION

The cryopreservation of ovarian tissue containing primordial follicles is a new route in research on the conservation of female gametes. Ovarian tissue is used in human medicine such as primarily to restore fertility in women who have undergone chemotherapy or radiotherapy. This class of treatments is used not only for malignant diseases like cancer but also in severe autoimmune pathologies such as rheumatoid arthritis (Demirci *et al*., 2003). Cryopreservation of spleen and lymph nodes as a source of mononuclear cells used for the development of monoclonal antibody producing hybridoma cells by efficiently using hybridoma technology for the successful generation of monoclonal antibody producing hybrid cells (Sogut *et al*., 2011).

OVERVIEW OF INTERLEUKIN-2 (IL-2)

IL-2 is a polypeptide that shows hormone like activities especially the clonal expansion of antigen specific cytotoxic T cells. IL-2 is a type of cytokine immune system signaling molecule which is a leukocytotrophic hormone that is instrumental in the

body's natural response to microbial infection and in discriminating between non-self and self antigens. IL-2 is normally produced by the body during an immune response (Cantrell and Smith, 1984). Dendritic cells are unique antigen-presenting cells capable of triggering NK cell effector functions and priming naive T cells and inducing IL-2 production (Schartz *et al*., 2005). IL-2 mediates its effects by binding to IL-2 receptors which are expressed by lymphocytes, the cells that are responsible for immunity (Meuer *et al*., 1982). Initial contact of a new foreign antigen with a naive T-cell initiates the cognitive phase. This is immediately followed by the release of lymphokines (IL-2 and/or IL-4) that function as auto-crines to stimulate growth, proliferation and differentiation of the newly stimulated T-cell during the activation phase to produce clones of effector and memory T-cells. Effector T-cells have many functions including the triggering of antibody production by B-cells, activation of monocytes and macrophages, the stimulation of inflammatory reactions, further differentiation into cytotoxic T-cells.

After antigenic presentation, antigens bind to the TCR and stimulate the secretion of IL-2 and the expression of IL-2 receptors IL-2R (Sileghem *et al*., 1989). The IL-2/IL-2R interaction stimulates the growth, differentiation and survival of antigen-selected cytotoxic T cells via the activation of the expression of specific genes (Stern and Smith, 1986). After mitogenic stimulation, IL-2 is secreted by both $CD4^+$ and $CD8^+$ T cells (Meuer *et al*., 1982). IL-2 is enriched at the site of DC and T cell interaction and promotes allogeneic T cell proliferation (Schartz *et al*., 2005). IL-2 has been found to be similar to IL-15 in terms of function. Both cytokines are able to facilitate production of immunoglobulins made by B cells and induce the differentiation and proliferation of natural killer cells. The primary differences between IL-2 and IL-15 are found in adaptive immune responses. IL-2 participates in maintenance of T-Regulatory cells and reduces self-reactive T cells and IL-15 is necessary for the maintenance of highly specific T cell responses by supporting survival of $CD8^+$ memory T cells (Waldmann, 2006).

CLINICAL ASPECTS OF IL-2

IL-2 has been tested in many clinical trials for immunomodulation for the treatment of cancers, chronic viral infections and as adjuvants for vaccines. Many of the immunosuppressive drugs such as corticosteroids, cyclosporine and tacrolimus used in the treatment of autoimmune diseases and organ transplant rejection, respectively work by inhibiting the production of IL-2 by antigen-activated T cells. Sirolimus blocks IL-2R signaling thereby preventing the clonal expansion and function of antigen-selected T cells. Thus, IL-2 plays an important role in the pathogenesis of autoimmune, immunodeficiency and lymphoproliferative diseases (Bachmann and Oxenius, 2007).

IMMUNE RESPONSE OF VARIOUS SPECIES OF MICE

Mice represent a wide range of genetic origins. Most of the mice strains used in genetic, physiological and immunological studies have seen produced transgenically studies such as C57BL/6J, BALB/c, SJL/J, RIIIS/J C3H/HeJ, PWD/PhJ, WSB/EiJ, etc. used for immunological studies. The lymphocyte population in these species is analyzed and found that RIIIS/J and C57BL/6J female mice showed the highest percentages of peripheral blood lymphocytes while C3H/HeJ, PWD/PhJ and WSB/EiJ mice has been found that the lowest proportion of lymphocytes. Balb/c mice has been found the intermediate and PL/J has been found the high proportions of lymphocytes (Petkova *et al*., 2008).

Balb/c and C57BL/6 are widely used strains for the study of immune related diseases because of Th1/Th2 shift response pattern. Th1 and Th2 are the type of T-helper cells. Th1 (T helper 1) response is an essential component of immune related diseases and produces high amount of IL-2, IFN-γ and IL-12 and low amount of IL-4 and IL-5. Th2 response is known as counter-regulatory to Th1. Th1 response and Th2 responses are mutually antagonistic. Th2 mediators such as IL-4 and IL-5 show inhibitory response on Th1 by preventing disease related pathways and by inhibiting mature Th1 cell response (Capsi, 2002). The C57BL/6 mice induces Th2 type response (IL-4, IL-5 and IL-13) while Balb/c mice induce Th1 (IL-2, INF-gamma) type response naturally but after expression of mitogen C57BL/6 mice induces Th1 response while Balb/c mice induces Th2 responses (Morokata *et al*., 1999). Balb/c is more susceptible to intracellular parasite infection has a higher tumor incidence and is more resistant to the induction of autoimmune diseases, as compared to C57BL/6 mice. The frequency of T regulatory cells in the thymus and peripheral lymphoid organs of Balb/c mice are higher than in C57BL/6 mice. It has been reported that lymphocyte cells from C57BL/6 mice produce lower levels of IL-2 than lymphocyte cells from Balb/c. Secretion of IL-2 from lymphocyte cells are associated with depletion of T regulatory cells (also known as T suppressive cells) in the mouse thymus and periphery lymph node in C57BL/6 mice. Balb/c mouse T cells produce higher levels of IL-2 and have a higher proliferative response of T regulatory cells (Chen *et al*., 2005).

OVERVIEW OF CONCANAVALIN A (CON A)

The Con A (*Canavalis ensiformis*) has extensively been used as a T-cell-specific mitogen (Sharon, 1983). The protein Con A is a lectin found in the jack bean it crystallizes into two fractions that is Concanavalin A and B. Con A is soluble in water and interacts with glucose and mannose-specific carbohydrate structures. It shows the agglutination of red blood cells, so has been called a hemagglutinin (Ballerstadt *et al*., 2006). The mechanisms by which Con A exerts its biological effect on T lymphocytes may depend in part on the organization of its subunits, the number and type of binding sites and valance for lymphocytes surface binding (Wands *et al*., 1976). Con A binds to the lymphocyte membrane specifically through its sugar-binding sites. The average maximum number of Con A molecules that bind to lymphocytes was about 5×10^6/cell. Con A requires an initial interaction of the mitogen with the surface of the cells that have an intrinsic capacity to be activated by this compound (Betel and Knor, 1972). Internalization of Con A is not required for lymphocyte activation and Con A binding to the surface membrane of lymphocytes appears sufficient to induce blastogenesis (Black *et al*., 1987). T-lymphocyte activation by antigens, mitogens is dependent on macrophages or is enhanced by these cells. One major function of macrophages in lymphocyte activation appears to be the uptake and presentation of antigens. This function requires that the macrophages and the lymphocytes be histocompatible. However, macrophage-dependent lymphocyte activation is also mediated by factors released from these cells such as lymphocyte activating factor (Knop, 1980). Microplate culture system has been used to standardize mouse lymph node lymphocyte responses to Con A. The lymphocytes stimulation by Con A depends upon the effects of microplate well-shape, cell concentration, mitogen dose, culture time, buffering system and initial pH of culture on lymphocyte transformation (Thorpe *et al*., 1976).

Lectins are emerging as bioactive plant proteins to be used in biomedicine, especially for the potential cancer treatment. Several lectins have been found to possess anti-cancer properties. They are used as therapeutic agents, preferentially binding to cancer cell membrane causing cytotoxicity via inducing apoptosis, autophagy or necrosis and inhibiting the tumor growth. Furthermore, they could simultaneously activate the immune system, stimulate the cytokine production inside the tumor mass and the recruited lymphocytes would participate in the eradication of the tumor (Huan and Chang, 2009). *In vitro* effect of Con A in cell cultures includes mitogenicity, teratogenicity and cytotoxicity. Con A has the ability to induced mitogenesis (an induced mitotic cellular division) in T cells which have been associated with cytokine expression and secretion. Con A has also been found to bind to surface glycoproteins and glycolipids of many cell types including leukocytes, keratinocytes, hepatocytes and a large number of transformed and non-transformed cell lines. The teratogenic effect of Con A includes decreased in viability of rat embryonic cell, yolk diameter, crown-rump length and increases in the incidence of morphological abnormalities characterized by neural tube defects. The cytotoxic effect of Con A includes decreased cell viability in lymphocyte cell cultures by mechanism of lectin-dependent cellular cytotoxicity of antigen specific, cloned cytotoxic T lymphocyte cell lines and induced T cell activation by specific interaction with a cell surface antigen receptor complex and the T cell was activated, it grew and matured into cytolytic effector cells (Ballerstadt *et al*., 2006).

Con A showed variety of biological effects including mitogenesis, cytotoxicity, hepatotoxicity and teratogenicity in cell cultures and in animals. Con A-induced stimulation of DNA synthesis in animal cells and has potency as an immunostimulant and effective model agent for hepatic failure (Ballerstadt *et al*., 2006). Hepatic injury by Con A is characterized by apoptotic cell death *in vivo* effects. Con A increases the level of cytokines like TNF-α, IL-2, granulocyte macrophage-colony stimulating factor and IFN-γ in the circulation of the mice (Gantner *et al*., 1995). Con A have a potent anti-hepatoma effect. Con A preferentially binding to cancer cell membrane causing cytotoxicity via inducing apoptosis, autophagy, necrosis and inhibiting the tumor growth. Con A also activates the immune system and stimulates the cytokine production inside the tumor mass and the recruited lymphocytes in the eradication of the tumor (Huan and Chang, 2009). Con A is also an effective for establishment of arthritis model in rat paws (Delitheos and West, 1976). Low concentration of Con A has profound insulin-like effects on metabolic processes of isolated fat cells. Con A also interacts directly with the surface receptors of fat cell as insulin and provide a better understanding of the mechanism of action of insulin (Katzen *et al*., 1981). Another study showed that Con A stimulated mouse splenocytes and produced prolactin like activity in culture medium *in vitro* effects (Gala and Rillema, 1995).

OVERVIEW OF IMMUNOLOGICAL TECHNIQUES: ELISA AND FLOW CYTOMETRY

ELISA and Flow Cytometry are immunological techniques which are used for the counting of CD[4] T cells.

Flow cytometry specifically identify and count individual $CD4^{+}$ T lymphocytes on the basis of the presence of cell-surface CD^{4+} antigen and other cellular characteristics. The ELISA measures total CD^{4} T cells antigen in sample. Some of the CD^{4} antigen detected by ELISA originates from CD^{4+} lymphocyte surfaces but this cannot be distinguished from CD^{4} antigen derived from monocytes or eosinophils from intracellular sources or from soluble antigen originally present in sample. Although, flow cytometry with conventional apparatus is currently too expensive for routine CD^{4+} T-lymphocyte counting in sample (Shapiro *et al*., 2004). ELISA is an enzyme-based immunoassay method which is useful for measuring antigen concentrations. It is a powerful technique is used to measure cytokines in biological samples and cell culture supernatants (Ledur *et al*., 1995). ELISA techniques are widely used in forensic toxicology for initial drug identification purposes and predominantly designed for use with urine. When urine is not available, immunoassays designed for blood and other specimens such as bile and liver homogenates can be screened. However, prior treatment with a solvent or precipitation of proteins is required. Solvent extraction provides the highest sensitivity and reduces interferences from other components in the specimen (Moore *et al*., 1999).

It has also found applications in the food industry in detecting potential food allergens such as milk, peanuts, walnuts, almonds and eggs. ELISA tests are also used as in *in vitro* diagnostics in medical laboratories. The other uses of ELISA are detection of Mycobaterium antibodies in tuberculosis, detection of rotavirus in feces, detection of hepatitis B markers in the serum, detection of enterotoxin of *E. coli* in feces and detection of HIV in blood samples (Griffin *et al*., 2005). It is due to the amplifying potential of enzyme labels, immunoassays that use enzyme-conjugated antibodies have become increasingly popular because of their high specificity and sensitivity (Crowther, 1995). Cytokine sandwich ELISA is sensitive enzyme immunoassays that specifically detect and quantitate the concentration of soluble cytokine and chemokine proteins. The basic cytokine sandwich ELISA method makes use of highly-purified anti-cytokine antibodies (capture antibodies) which are noncovalently adsorbed (coated-primarily as a result of hydrophobic interactions) onto plastic microwell plates. After plate washings, the immobilized antibodies serve to specifically capture soluble cytokine proteins present in samples which are applied to the plate. After washing away unbound material, the captured cytokine proteins are detected by biotin-conjugated anti-cytokine antibodies (detection antibodies) followed by an enzyme-labeled avidin or streptavidin stage. Following the addition of a chromogenic substrate, the level of colored product generated by the bound, enzyme-linked detection reagents can be conveniently measured spectrophotometrically using an ELISA-plate reader at an appropriate optical density (Crowther, 1995). Therefore, cytokine sandwich ELISA can discriminate between cytokines that can have overlapping biological functions which are not resolvable in a bioassay. Sandwich ELISA for measuring cytokines and their receptors have become increasingly important as diagnostic tools and for monitoring therapeutic regimens, e.g., biological response modification regimens utilizing recombinant cytokine proteins. In the latter cases, highly optimized sandwich ELISA kits designed to minimize interference or nonspecific reactivities presented by patient samples is highly desirable (Abrams, 1995).

CONCLUSION

Lymph nodes are secondary type of lymphoid organ and involve in immune mediated pathways by activating T and B cell responses with the help of different antigens and mitogen. Concanavalin A is a plant mitogen which is used for the proliferation of T-lymphocytes because T-lymphocyte is identified as effector cell of Concanavalin A. *In vitro*, Concanavalin A stimulation of mouse lymph node cells culture induces the release of IL-2 in cell culture and release of IL-2 is measured by ELISA kits and flow cytometry.

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