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Effects of AHCC on the Interaction between T and B Lymphocytes

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EFFECTS OF AHCC ON THE INTERACTION BETWEEN T AND B LYMPHOCYTES

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Professor Alamelu Sundaresan, Ph.D., Advisor

AHCC is a beta-glucan derived from the shiitake mushroom. It is used as an immune supplement and is known to boost the immune system. The mechanisms involved are increased lymphocyte proliferation and reduction of inflammation. The effects have been well studied in T cells. However, factors produced by T cells that affect B cells have not been studied. In this study, we explored the gene products stimulated by the AHCC exposure in T lymphocytes which play a role in B cell function. Data from a prior gene array experiment were used to delineate the effects of AHCC on the interaction between T and B lymphocytes.

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LIST OF ABBREVIATIONS

AHCC	Active Hexose Correlated Compounds
FANCD2	Fanconi anemia, complementation group D2
LRRC8A	Leucine-Rich Repeat Containing 8 VRAC Subunit A
IL1R2	Interleukin 1 Receptor Type 2
RNF128	Ring Finger Protein 128
LAT	Linker for activation of T cells
TNFSF14	Tumor Necrosis Factor Superfamily Member 14
VSIG4	V-Set and Immunoglobulin Domain-Containing Protein4
HCST	Hematopoietic Cell Signal Transducer
CD1A	Cluster of Differentiation 1a
MLLT11	Myeloid/Lymphoid or Mixed-Lineage Leukemia; translocated to, 11

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CHAPTER 1

INTRODUCTION

The only cells in the organism to be able to recognize and respond specifically to each antigenic epitope are the T and B lymphocytes (cells). B cells make the antibodies and are responsible to transform them into plasmacytes. [Cano et al., 2013] The immune system consists of humoral immunity that depends on the B cells and Cell-mediated immunity that depends on the T cells which protects the body from infections. The immune system has good and bad aspects that fight against invading foreign materials. The immune system can be linked to genetic makeup and covers a way for novel prevention and treatment measures to work against infectious and immune-mediated diseases. The innate and adaptive immune responses work together to eliminate the pathogens. The adaptive immune response is highly specific in comparison to the innate immune responses which provide long-lasting protection against infection [Alberts et al., 2002]. This unique complicated and detailed system consists of two defense mechanisms: the innate system, which involves the nonspecific immune response, and the adaptive immune system, which involves antigen-specific immune response. Whole different types of cells and their functions give scientists the knowledge of how the cells behave under different physiological stress that will also help in the study of immune augmenting agents. Mushrooms are of great beneficial effects which provide a very good effect on health and

illness. Mushrooms are considered to boost immunity through traditional medicines practiced in different parts of the world [Shin et al., 2019].

From a morphological point of view, T and B lymphocytes are indistinguishable because they are both small cells (8–10 microns in diameter). Both T and B lymphocytes have large nuclei containing heterochromatin and cytoplasmic boundaries with few mitochondria, ribosomes, and lysosomes. After activation by antigenic stimulus, they can expand, thereby increasing the number of their cytoplasm and organelles. Lymphocytes also present receptors for antigen (Ag) recognition. These are TCRs and BCRs respectively. [Cano et al., 2013].

Lymphocytes were used in this study because they represent the prominent specificity of the adaptive immune response. They are abundant in the human body, especially in the blood, lymph, and lymphatic organs such as the thymus, lymph nodes, spleen, and cecum. The major role of lymphocytes in adaptive immunity has not been established for a long time, despite the large population of the human body. Experiments were performed on strongly irradiated rats and mice to kill most white blood cells, including lymphocytes, making it difficult to elicit an adaptive immune response intracellularly. [Alberts et al., 2010] Both innate and adaptive immune systems work together to defend against harmful foreign pathogens.

During maturation, T Cells have receptors called T Cell Receptor that recognizes a specific Ag. TCR is a multi-protein complex consisting of two variable antigen-binding chains, αβ or γδ, with the invariant accessory proteins (CD3γε, CD3δε, and CD247 ζζ chains) required to initiate signal transduction as TCR binds to an Ag. [Wucherpfennig et al., 2010]

A successful immune function is the coordinated interactions between different cell types. An example of this is orchestrated cooperation between helper T cells and B cells that occurs between T-cell-dependent antibody responses. While these processes show rapid immune defense against infection, treatment with AHCC enhances the immune system.

On the immune system, the effects of mushrooms could stem from bioactive polysaccharides such as beta- (β-) glucans or polysaccharide complexes in mushrooms in that these molecules appear to affect innate and adaptive immune responses [Alberts et al., 2010] AHCC® is a standardized extract of cultured shiitake or Lentinula edodes mycelia (AHCC®) which incorporates aggregate of vitamins which include oligosaccharides, amino acids, and minerals acquired through the liquid lifestyle manner of shiitake mycelia. [Roman et al., 2013] AHCC is produced with the aid of using Amino Up Co., Ltd. (Sapporo, Japan) beneath neath the trademark "AHCC®." The shiitake mycelia used for AHCC® are cultured in a liquid medium wherein the mycelia proliferate and shape globular fungi in our bodies however now no longer fruiting our bodies. [Nogusa et al., 2009] Of the oligosaccharides in AHCC®, approximately 20% are α-1,4-glucans, of which a share is partly acylated, with an average molecular weight of around 5000 Daltons [Roman et al., 2013]. In immune cells of people and animals, the outcomes of AHCC® are said in vitro and in vivo studies, suggesting the assistance assist of its supplementation in protecting the body from infections and malignancies thru modulating the immune system [Aviles et al., 2004]. AHCC complements the immune system's feature, which can bring about multiplied resistance of the host to distinctive infections [Belay et al., 2015].

The potential effects of AHCC® on T cell immunity and B cell immunity may be biologically important in the development of an immune response to antigens which is supported by studies reporting increased proliferation of B cells and T cells proliferation with the treatment with AHCC. T cells play an important role in the host's defense against microorganisms and malignancies, that are a part of the adaptive immune response [Lee et al., 2012]. B cells and macrophages are promoted by CD4+. T cells are T helper cells (Th) in that they secrete cytokines and express costimulatory molecules [Murphy et al., 2010]. Cytotoxic CD8+ T cells, can kill infected or tumor cells, armed with the cytotoxic molecules perforin and granzymes [Cui et al., 2010]. Mushroom extracts, especially polysaccharides, have been reported to promote an immune response against tumors by affecting the function of T cells and B cells [Meng et al., 2016].

AHCC boosts immunity. It shows beneficial effects on B and T cell proliferation. AHCC enhances immunity. In this test, we studied the genes beneficial to immune cells and AHCC regulated. Another important part of this study is to find out factors that are produced because of the AHCC proliferation that facilitate the interaction between B and T cells. However, factors produced by T cells that affect B cells have not been studied. In this study, we explored the gene products stimulated by the AHCC exposure in T lymphocytes, which play a role in B cell function. Data from a prior gene array experiment was used to delineate the effects of AHCC on the interaction between T and B lymphocytes.

CHAPTER 2

LITERATURE REVIEW

The immune system shields the body against infection and from the foreign invasion of harmful microorganisms through self-defense mechanisms. The immune system has two different types of defense mechanisms: Innate Immunity and Adaptive Immunity.

Innate immunity is the first line of defense against intruding pathogens. Adaptive immunity provides antigen-dependent and antigen-specific types of immunity. Both work together to protect against disease or illness [Marshall et al., 2018].

White blood cells (WBCs) are special cells, also known as the soldiers of the body, that make up the overall immune system, which functions through its specialized cell members of the body. Granular WBC, which is primarily involved in the body's immune response, is derived from the bone marrow and is a lymphocyte found in body fluids, blood, and lymphocytes.

The immune system plays an important role in the defense of the host as it is a vast network of different organs, cells, and proteins in the body. When pathogens and viruses from outside invade the human body, immune systems such as innate and adaptive immunity step up to protect against pathogens. Immune lymphocytes are granulocytes such as eosinophils, neutrophils, and basophils. Immune lymphocytes are B cells, T cells, and natural killer cells. And antigen-presenting cells, which are macrophages,

monocytes, dendritic cells, etc. are interconnected by defense functions. However, various factors such as aging, stress, and malnutrition are involved in cell function.

T cells and B cells are the main types of lymphocytes. Both are involved in the immune response. Antibodies are produced by B cells and that can destroy invading microorganisms such as bacteria and viruses, while T cells produce cytokines and are also the direct fighters of foreign intruders.

Humoral Immunity is provided by the B cells that mature in the bone marrow, and cell-mediated immunity is provided by the T cells that mature in the Thymus. The adaptive immune response has the main components of B cells and T cells. T lymphocytes have a variety of functions because normal macrophages are derived from myelomonocyte precursors that migrate to tissue through the blood. [Wing et al., 1977]. Innate Immunity also presents NK cells or natural killer cells, a type of cytotoxic lymphocyte. Below are the other types of cells that are a part of the immune system.

Neutrophils are granulated white blood cells that cause inflammatory effects. They are in a large amount in total WBCs. Monocytes are granular-free WBCs accounting for a very less amount of the total WBC count and are the largest. These are phagocytic. Monocytes are made in the bone marrow, transferred to the blood, and circulated for 2-3 days to become macrophages or dendritic cells (antigen-presenting cells). Eosinophils are active in allergic reactions, and granular white blood cells promote inflammation. They regulate the inflammatory response and trap and kill cells infected with bacteria and parasites. Basophils are granular white blood cells that make up less than 1% of all white cells that cause allergic reactions because they contain n histamine. Macrophages occur through a specific immune process. Macrophages are activated when, in addition to

specifically sensitized lymphocytes, lymphocytes are exposed or exposed to previously sensitized substances such as bacterial endotoxin [Wing et al., 1977]. Cell-mediated immunity response functions using antigen-presenting cells and T-cell lymphocytes, which do not use antibodies. It typically occurs in response to body cells that are infected by bacteria, viruses, or fungi. It also gets activated by the presence of cancer cells in the body.

Cell-Mediated Immune Response Contact between lymphocytes and antigen occurs either at the site of infection or in lymph nodes draining the peripheral areas, where the organism has invaded. -IX lymph nodes, T lymphocytes localize to and are sensitized in periarteriolar areas which are referred to as thymus-dependent areas. Two types of antigens stimulate T lymphocytes but not B lymphocytes: the first is certain small molecules such as aminobenzoate-N-acetyl tyrosine or some synthetic peptide copolymers of tyrosine or glutamic acid; which are bigger. Those antigens most likely to induce sensitization of T lymphocytes in vivo are proteins found on the membranes of living cells. Antigens on dead cells or antigens alone are less effective [Wing et al., 1977]. CMI comes under the acquired or adaptive type of immune response, which means that memory cells are developed in the body after exposure to pathogens. The first contact with a pathogen produces a primary response that is decreased in intensity. CMI mostly kills cancer-affected cells. Subsequent exposure to the same pathogen produces a highly intensified secondary response. This shows that our body appears to have a memory of the first encounter. It is specific and is slower in action than innate immunity. CMI actions are controlled mainly by the action of T-cells. CMI for short is produced by specific cells of our bodies which is the third line of defense mechanism which is different from the immunity provided by antibodies, and it

gets initiated when the first line of defense (innate immunity) and the second line of defense (nonspecific resistance) fails to protect the body.

T helper cells, T killer cells, T suppressor cells, and T memory cells are the four types of T cells. T-helper cells help other cells like macrophages and B cells by stimulating immunity. T-helper cells recognize MHC-II with the help of the CD4 receptors present on the surface of the cells. Cytotoxic cells /T-killer cells: Also known as cytotoxic cells as they kill the infected cells and do not normally damage the uninfected cells. The CD8 receptor assists the recognition of MHC- II complex forms. The perforins produced by these complexes cause cellular lysis. T- suppressor cells are a subpopulation of T cells; this cell suppresses the action of T cells. It helps to stop autoimmunity in the body. T-memory cells function when there is a second exposure to an antigen. Memory T cells act faster to convert into effector cells. MHC is on the cell surface which helps the immune system find the outside substances. The major histocompatibility complex is present in every cell in the body except red blood cells. There are two types of major histocompatibility complex proteins. MHC I help the body distinguish between the cells of the body and foreign cells or pathogens. Immune cells called the antigen-presenting cells (APCs), such as lymphocytes, dendritic cells, and macrophages, have another set of a protein called MHC II along with MHC I. The MHC I molecule displays an antigen on the surface of this cell, but phagocytosed microorganisms appear on the surface of MHC II.

CD8 Cytotoxic **and CD4 T Cells**

T Lymphocyte/ or CTL for short is also called the CD8 T cell. Its predominant characteristic is to break inflamed cells earlier than they launch mature parasites. If the

immune system can destroy infected cells before they release new parasites, it will be much easier to control the infection, cells are always displaying sample peptides of internal proteins on the outside of the plasma membrane. The cells show those peptides so that CD8s and different immune cells can screen them and spot a pattern of what's within the cell. However, the molecule that presents antigens on a cell's surface is none other than the MHC 1 molecule, and that nl killer cells kill cells that do not specifically express MHC 1 molecules on their plasma membrane. So, the virus may evade the CD8s only to be destroyed by natural killer cells. CD8 T cells have CD8 glycoprotein on their cell membranes that act as the antigen receptor. If the antigen receptor of the CD8 T cell binds to an antigen presented on the cell surface, the CD8 will kill the cell and that DNA within the cell is likewise destroyed to that cell would not release viable parasites while it is killed.

The nature and concentration of Antigen determine the differentiation of CD4+ T cells into cell phenotypes. Activation and the type of APC, the microenvironment of the cytokine accompanies the antigen presentation/ and other variables. The cell express CD4 which is converted to T helper (Th) which functions as the cell to produce cytokine and stimulate B cells to generate Abs. Th1, Th2, Th12, and T regulatory cells (Treg) are the four types of T helper cells to secrete different types of cytokines [Cano et al., 2013].

Immune System serves to quick reply to foreign cells to combat infection, against the virus, or protect the body from bacteria. Innate and Adaptive Immunity are involved in the key mechanism to protect the body from foreign pathogens. Acquired immunity, also known as adaptive immunity, makes use of T-cells and B-cells whilst invading organisms slip through that first line. These cells take longer to develop/ due to the fact their behaviors are our experiences. But they have longer self-life than immune cells. An adaptive system

remembers the outside particles after the first infighting and fight when those particles re-enter the body. is for how vaccines work—using a small, harmless amount of protein from disease. The immune system recognizes if the pathogens already entered the body.

The primary and secondary organs involved in the complex development of lymphocytes are the B-cells and T-cells. In most cases, the bone marrow and the thymus are the organs that generate B- and T-lymphocytes.

B cells and T cells interaction

B cells and T cells work together and produce specific antibodies against invading pathogens. Antigen-specific T helper cells and antigen-specific B helper cells work to form an antigen bridge that functions to bind an antigen molecule (the 'hapten'), exactly when T behaves at the same time to find another determinant (the 'carrier'). Helper T cells associate with antigen-presenting cells (APC), which ideally receive and process antigens, and this interaction, like that of helper T cells, with other cells. precise B specifically, limited by-products encoded by the major histocompatibility complex (MHC). While conventional APCs such as macrophages do not have binding specificity for antigens, B cells possessing immunoglobulin receptors that are cloned specific antigens can be predicted to decorate the ability to deliver I-antigen to T cells. These findings are hard to reconcile with the simple 'antigen bridging' interactions due to the fact it is difficult to describe the two molecular complexes (treated antigen plus MHC molecule) on the surface of APC may resemble a three molecular complex (surface immunoglobulin binding antigen plus MHC molecule) on the surface of B-cell. [Lanzavecchia et al., 1985].

Both B cells and T cells are derived from the same cell, HSC (hematopoietic stem cells are derived from the bone marrow). B cells travel throughout the body and the spleen. On the surface of the B cells, there are B cell receptors that have the function of binding to antigens. When the antigen binds to the antigen receptor on B cells, then the antigen is swallowed by the receptor-mediated endocytosis. Helper T cells recognize and bind to MHC II. When bound, it releases lymphokines. Plasma cells release specific antibodies against the antigens first bind to the B cells.

Helper T cells (CD4) bind to MHC II on the surface of B cells, and this binding releases lymphokines. MHC class I presents CD8 T cells on the surface of the infected cells. T cells secret cytotoxins that initiate programmed cell death. When binding occurs, the cell expresses a ligand called the Fas ligand that binds to the Fas molecule.

Interactions between B and T cells require Ag presentation to T cells and continuous interaction between cytokine, chemokine, CD28/B7, and receptor/ligand pairs belonging to TNF/TNFR superfamily., Trigger the required transcriptional program for each cell type. B cell activation and differentiation require signal transduction by follicular helper (Tfh) cells at multiple spatially splendid checkpoints to ensure the levels and maximum antibody response. The cells coordinately express specific molecules, co-localize with the GC B cells in the follicular and secondary lymphoid tissues, and provide stage-specific helper alerts to cognate B cells. This technique is essentially regulated by unique transcription elements and extrinsically by follicular T- regulatory cells (Tfr). The importance of molecular interactions between B and T cells is evidenced by evidence that immunodeficiency and autoimmune disease expand as they affect these interactions. [Stuart et al., 2010]

The reduction of the activation of the immune system is Immunosuppression. Immunosuppressants are the principal approach for consciously inducing immunosuppression, in certain circumstances, immunosuppressive drugs primarily goal hyperactive additives of the immune system.

Mushrooms were taken into consideration to have viable useful consequences on fitness and sickness for decades via way of means of conventional drugs practiced in exceptional areas of the world. Although the precise organic mechanisms underlying such consequences are but to be elucidated, extracts from a set of mushrooms are commercially used as nutritional dietary supplements and useful ingredients in fitness situations probable related to immune dysregulations that consist of infections, and inflammatory diseases, and malignancies.

The consequences of mushrooms at the immune device should stem from bioactive polysaccharides consisting of beta- (β-) glucans or polysaccharide complexes in mushrooms, in that those molecules seem to influence innate and adaptive immune responses. Also, the research found the activation of Natural killer (NK) and T cells via way means of using alpha- (α-) glucans extracted from fit for human consumption mushrooms like Tricholoma matsutake and maitake assisting the implication of α-glucans in regulating the immune machine. [Sato et al., 2017]

Active hexose correlated compound (AHCC), an extract prepared from mycelia of the basidiomycete mushroom Lentinula edodes, has obtained unique benefits, which have been used to revitalize the immune reactions of the host. AHCC is a nutritional supplement that is used in a variety of commercial ways and constitutes a mixture of polysaccharides, amino acids, and minerals.

AHCC has no chemical effects, which constitutes a large amount of nutritional supplement. AHCC contains a mixture of polysaccharides, amino acids, and minerals. It is oligosaccharides that have a very low molecular weight (5 KDa) containing partially acetylated forms of α-1,4-glucan. Consumption of AHCC for at least 30 days boosts CD4+ and CD8+. [Yin et al., 2010]

CHAPTER 3

DESIGN OF THE STUDY

RNeasy mini kit was purchased from Qiagen, USA (Cat. Number 74104, Inverted Microscope: Eclipse TS100(Nikon)). RNA isolation was done as per the RNA isolation instructions obtained from the RNeasy mini kit purchased from Qiagen. RNA Analysis by Illumina. For the identification of differently expressed genes between different conditions or tissues, RNA-seq has become the most used alternative to microarrays. RNA isolation by Illumina allows for high coverage of the genome and also detects weakly expressed genes [Hass et al., 2015]. Data were obtained from previously performed experiments. GenomeStudio software (Illumina, CA) was used to analyze the data. GenomeStudio and Ingenuity Pathway Analysis (Ingenuity Systems, Inc.) software, respectively were used for performing clustering and pathway analysis [Loretta et al., 2015].

RNA isolated from harvested cells is subjected to a gene array analysis. The cells harvested were analyzed and subjected to the Illumina platform. To characterize the unique phenotypic and proliferation phenomena seen with AHCC treatment in resting lymphocytes compared to control, the cells harvested were analyzed and subjected to the Illumina platform. From the gene array data obtained, A group of significant genes FANCD2, LRRC8A, IL1aAR2, RNF128, LAT, TNFSF14, VSIG4, HCST, CD1A, MLLT11 responsible for cell proliferation, cell adhesion, and/ or receptor signaling, triggering of T-lymphocytes, as well as signal transduction, were identified.

RNA sequencing (RNA-Seq) is revolutionizing the look of the transcriptome. A fantastically touchy and correct device for measuring expression throughout the transcriptome, it is giving a vision to the researchers with visibility into previously undetected modifications going on in sickness states, in reaction to therapeutics, beneath specific environmental conditions, and throughout a wide variety of different study purposes.

RNA-Seq enables the detection of transcript isoforms, gene fusions, single nucleotide variants, and other features without the limitation of prior knowledge. Hence allows researchers to detect both known and unknown features of the assay.

The RNA isolated from harvested cells was subjected to gene array analysis. From the gene array data that was obtained, a group of significant genes FANCD2, LRRC8A, IL1R2, RNF128, LAT, TNFSF14, VSIG4, HCST, CD1A, MLLT11 were selected for gene array analysis. The expression profile of genes associated with B lymphocytes and T lymphocytes were analyzed. Gene array data for AHCC-treated lymphocytes on 100µm/ml and 750µg/ml was obtained from previously performed experiments [Olamigoke et al., 2015] which were further compared, and their fold change difference of the upregulated genes were studied.

String analysis is a tool that performs searches and visualizes the results in their genomic context. The genomic ally-associated genes and their functions can be found with this tool. [Snel et al., 2000] STRING is a database of known and predicted protein-protein interactions. The interactions in the string analysis give the association between the physical and functional association of the genes/ and their interactions with other primary databases. The STRING database collects and integrates all publicly available sources of

protein-protein interaction information and provides computational predictions. String analysis aims to achieve a comprehensive and objective global network, including direct (physical) and indirect (functional) interactions [Szklarczyk et al., 2019]. Here in this experiment, the gene-gene interaction was studied through string analysis. A group of significant genes associated with B lymphocytes and T lymphocytes was subjected to a string analysis. In a string analysis network, the nodes are proteins, the edges represent the predicted functional associations which may be drawn up to 7 different colored lines- these lines represent the existence of different seven types of evidence used in predicting the associations. The Red line indicates the presence of fusion evidence, the blue line- cooccurrence evidence, the green line- neighborhood evidence, the purple line- experimental evidence, the yellow line- text mining evidence, the light blue line- database evidence, and the black line- expression evidence [Szklarczyk et al., 2019].

Standard error was calculated based on the mean and the standard deviation and the standard error bars were included in the data. A "*P*" value less than 0.05 was considered significant and statistically significant. The upregulated and downregulated genes of interest were calculated based on a P value of less than 0.05. A P value less than0 .05 was considered significant, and a P value less than .10 but larger than .05 was considered to have the tendency. These analyses were performed using statistical software Stat View version 4.5. Results are expressed as mean + standard error.

CHAPTER 4

RESULTS AND DISCUSSION

Results showed that the immune competence was not altered in the cells with the treatment with the active hexose correlated compound group. It suggested that active hexose correlated compounds maintained total immunity. The Result shows that the change or increased proliferation of the cells associated with B cells and T cells when treated with AHCC. This clearly shows that AHCC is an immune booster. AHCC contributes to enhancing immunity. This experiment elaborated that there was a dose response-dependent increase of resting lymphocyte proliferation in response to AHCC treatment. These genotypic changes possibly involve signal transduction of cell adhesion molecules and adhesive cytokines and growth factors. From the gene array analysis, it was seen that certain genes responsible for T cell and B cell proliferation and differentiation were significantly upregulated (Tables 1 and 2).

Table 1: Regulation of Genes Associated with Both B and T Lymphocytes in T Lymphocytes Treated with Control and 750 µg/ml of AHCC

Genes associated with B and T lymphocytes	AHCC control (0 µg/ml)	AHCC CONC. (750 µg/ml)	Fold Change
FANCD2	55.73899	156.0903	+2.800379
LRRC8A	103.1918	257.9499	+2.499713
IL1R2	227.8257	540.6559	+2.373112

Table 2: Regulation of Genes Associated with T Lymphocytes in T Lymphocytes Treated with Control and 750 µg/ml of AHCC and the Fold Change with the Control.

Genes associated with T lymphocytes	AHCC Control (0 µg/ml)	AHCC (750 µg/ml)	Fold change
RNF128	43.22521	118.1648	+2.733701
LAT	372.5889	1010.14	+2.711138
TNFSF14	286.2363	746.0106	+2.606275
VSIG4	143.2714	369.24	+2.577207
HCST	740.2357	1890.678	+2.554157
CD1A	62.49257	156.1981	+2.499467
MLLT11	54.76192	130.1384	+2.37644

Note: Genes LRRC8A and IL1R2 are genes associated with both T and B lymphocytes.

Graphical Representation of Gene Array Data Associated with B and T Lymphocytes

Figure 1 is the graphical representation of genes treated with 750µg/ml of AHCC. Figure 2 is the graphical representation of genes treated with 750µg/ml of AHCC and fold change with control shows the graphical representation of the regulation of genes responsible for T lymphocyte proliferation, adhesion, differentiation, receptor signaling, and signal transduction leading to the activation of the immune system by evoking an immune response. It shows upregulated genes (750 µg/ml concentrations), and their corresponding signal intensity after a gene array analysis was performed. A P-value less than 0.05 was considered statistically significant. A P value less than 0.05 was significant, and a P value less than 0.05 but larger than 0.05 was considered to have the tendency.

These analyses were performed using statistical software Stat View version 4.5. Results are expressed as mean + standard error.

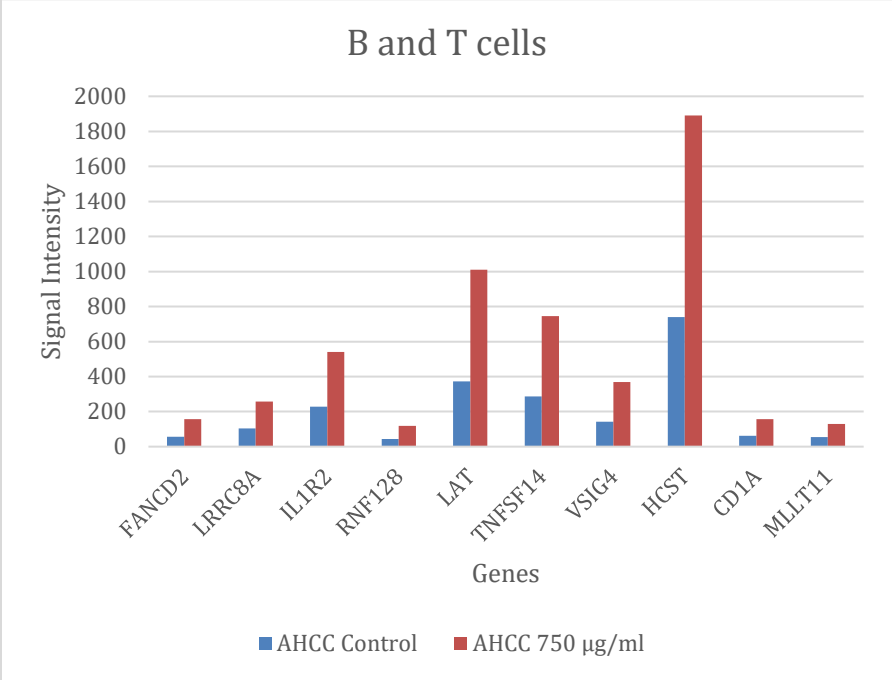

Figure 1: Graphical representation of Regulation of Genes Associated with both B and T lymphocytes in T lymphocytes Treated with Control and 750 µg/ml of AHCC. (P value less than 0.05 was considered statistically significant.)

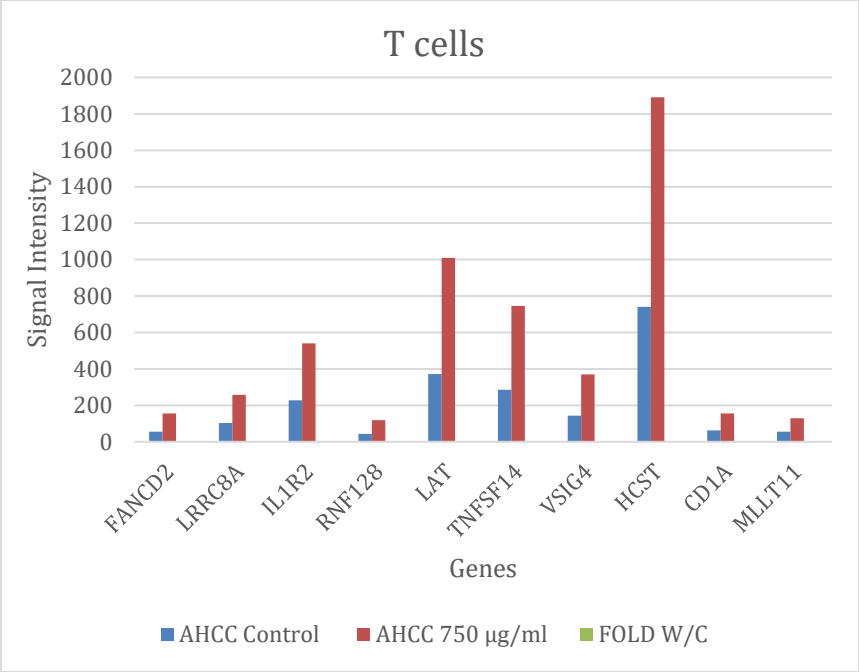

Figure 2: Graphical Representation of Regulation of Genes Associated with T Lymphocytes in T Lymphocytes Treated with Control and 750 µg/ml of AHCC and the Fold Change with the Control. (P value less than 0.05 was considered statistically significant.)

Gene Array Assay

After RNA is isolated from harvested cells, the RNA is subjected to a gene array analysis. The cells harvested were analyzed and subjected to the Illumina platform. The phenotypic and proliferation phenomena visible occur with AHCC treatment in resting lymphocytes compared to control, the cells harvested had been analyzed and subjected to the Illumina platform. From the gene array information obtained, A group of substantial genes FANCD2, LRRC8A, IL1aAR2, RNF128, LAT, TNFSF14, VSIG4, HCST, CD1A, MLLT11 accountable for cell proliferation, cell adhesion, and or receptor signaling, triggering of T-lymphocytes, in addition, to signal transduction, had been identified. Gene arrays are strong supports upon which a set of gene-particular nucleic acids were positioned

at defined locations, each via spotting and direct synthesis. In array analysis, a nucleic acid-containing pattern is categorized which allowed to hybridize with the gene-particular goals at the array. [www.genecards.org]

FANCD2 Gene

FANCD2 is the gene required for the renovation of chromosomal stability. It allows for promoting the correct and efficient pairing of the homologs at some point of strand breaks. It recombines and anneals the single strand withinside the restoration of double-strand breaks. Checks factor activation amongst DNA damage. Takes components withinside the S phase and Gs phase. Prevents breakage and lack of mis segregating chromatin at the end of cell division. Promotes BRCA2/ FANCD1 loading onto broken chromatin. Loading onto broken chromatin is promoted via way of means of BRCA2/FANCD1. Involved in B-cell immunoglobulin isotype switching. [Stelzer et al., 2016] The uptake of the drug cisplatin is executed via way of means of LRRC8A and LRRC8D which performs a key position as a transporter of immunoreactive cyclic dinucleotide GMP-AMP (2'-3'-cGAMP), an immune messenger produced in reaction to DNA virus withinside the cytosol: mediates each import and export of 2'-3'-cGAMP, thereby promoting transfer of 2'-3'-cGAMP to bystander cells. Particles containing LRRC8D The transport of 2'-3'-cGAMP is inhibited by the particles containing LRRC8D. Required for in vivo channel activity, collectively with at least a further own circle of a family member (LRRC8B, LRRC8C, LRRC8D, or LRRC8E); channel traits rely on the perfect subunit composition. Can shape purposeful channels via way of means itself.

Involved in B-cell development: required for the pro-B cell to pre-B cell transition. FANCD2 gene has a significant role in T-cell development. Involves in myoblast differentiation. Insulin-inspired glucose metabolism occurs through hyperpolarization and via VRAC activity oxygen intake occurs. Regulates glucose-sensing in pancreatic beta cells: Increase glucose sensitivity and insulin secretion develops as VRAC currents, generated in reaction to hypotonicity- or glucose-brought beta cell swelling, depolarize cells, thereby inflicting electrical excitation. Forms purposeful lysosomal VRAC channels in reaction to low cytoplasmic ionic energy conditions as a key position in lysosome homeostasis. Massive lysosome-derived vacuoles form because of lysosomal VRAC channels.

IL1R2 Gene

ILIR2 is the non- signaling receptor for IL1A, IL1B, and IL1RN. Interleukin 2 is only expressed in CD4+ T cells. In the process of activation of T cells, an important lymphokine is required. The membrane and secreted forms of ILIR2 bind IL1B and poorly IL1A and IL1RN. r IL1R2 binds to IL1B. IL1R2 plays a significant role in T cell activation, T cell proliferation, clonal expansion, and differentiation. T cell-specific and inducible expression. The secreted IL1R2 enlists secreted IL1RAP with enormous affinity; via secreted receptors. A transmembrane and as an alternate soluble form Interleukin 1 receptor, type 2, is conveyed in B and T cells. [Fishilevich et al., 2017].

RNF128 Gene

The RNF128 gene is an E3 ubiquitin-protein ligase containing the RING zinc-finger motif. The RNF128 gene arrests cytokine gene transcription, IL2, and IL4 transcription, thereby playing a major role in inducing the anergic phenotype, with the barriers to interleukin production. Transmembrane proteins help move ARPC5 and COR1A with 'Lys-48' linkages and 'Lys-63' junctions, respectively, resulting in damage and down-regulation of these cytoskeletal components. Reduced accumulation of F-actin at the immunological synapse. RNF128 acts on dorsal ectoderm patterning, and RNF128 sensitizes the ectoderm to reach nerve induction signals. It Involves inhibition of IL2 and IL4 transcription, thereby playing an important role in the endless state of T-lymphocyte inactivity. Antigenic stimulation is associated with blocking interleukin production. [Stelzeret al., 2016]

LAT Gene

LAT gene is Required for TCR (T-cell antigen receptor)- and pre-TCR-mediated signaling, each in mature T-cells and throughout the development. LAT Gene functions in the FCGR3 (low-affinity immunoglobulin gamma Fc region receptor III)-mediated signaling in natural killer cells and FCER1 (high-affinity immunoglobulin epsilon receptor)-mediated signaling in mast cells. Couples' activation of those receptors and their related kinases with distal intracellular occasions which include mobilization of

intracellular calcium stores, PKC activation, MAPK activation, or cytoskeletal reorganization through the recruitment of PLCG1, GRB2, GRAP2, and different signaling molecules [www.genecards.org].

TNSFS14 Gene

Cytokines bind to TNFRSF3/LTBR. Tumor necrosis factor superfamily member 14, plays an important role as a ligand for TNFRSF14 and its binding to the receptor TNFRSF6B shows its outcomes. T cells receive signals and proliferation and IFNG production are Up-regulated after T-cell activation. It is expressed in CD8+ tumor-infiltrating granulocytes, lymphocytes, and monocytes inducing apoptosis [Fishilevich et al., 2017].

VSIG4 Gene

The VSIG4 Gene functions as a phagocytic receptor that encodes a v-set and immunoglobulin-domain containing protein, a potent regulator of T-cell proliferation and IL2 manufacture. It also functions as a potent inhibitor of the alternative complement pathway convertases. VSIG4 is related to the B7 family of immune regulatory proteins.

HCST Gene

A transmembrane adapter protein that binds to KLRK1 and forms the activating receptor KLRK1-HCST in lymphocytes and bone marrow cells. This receptor plays a major role in inducing cytotoxicity against target-expressing cell surface ligands, including MHC class I chain-related MICA and MICB, and UL16-binding protein (ULBP). These ligands are up-regulated by stressful situations and pathological conditions, including viral

contamination and tumor transformation. PI3-kinase acts as a docking site for PIK3R1 and GRB2. Interactions cause calcium recruitment and activation of the PIK3R1, MAP2K/ERK, and JAK2/STAT5 signaling pathways. Both PIK3R1 and GRB2 are required for KLRK1-HCST-mediated full activation and ultimate target cell killing. In NK cells, KLRK1-HCST signaling complements cytokine production and begins via DAP12/TYROBP-related receptors. In T-cells, the TCR induction indicator is frequently stimulated. The KLRK1-HCST receptor plays a role in immune surveillance against tumors and is needed for the cytolysis of tumor cells. Cancer cells that do not express the KLRK1 ligands avoid NK cell-mediated immune surveillance.

T-cell receptor (TCR) ligation enhances KLRK1-HCST cell surface expression. Regulated by IL21/interleukin-21 in T-cells and NK cells [Fishilevich et al., 2017].

CD1A Gene

CD1A gene is an antigen-presenting protein that binds self and non-self lipid and glycolipid antigens and offers them to T-cell receptors on natural killer T-cells. Cell differentiation antigen CD1A (T6),49kDa, membrane glycoprotein, and B2 related diagnosed through monoclonal antibodies NA1/34, T6, VIT6, and Leu6. [Stelzer et al., 2017]

MLLT11 Gene

MLLT11 gene is involved in the regulation of lymphoid development. It drives multipotent hematopoietic progenitor cells towards a T cell fate, a cofactor for the transcription factor TCF7.

String Analysis

STRING is a database of recognized and expected protein-protein interactions. String evaluation is a device that plays searches and visualizes the outcomes of their genomic context. The gnomically related genes and their features may be located with this device. [Snel et al., 2000] STRING is a database of recognized and expected protein-protein interactions. The interactions withinside the string evaluation deliver the affiliation among the physical and functional association of the genes/ and their interactions with different number one databases. The STRING database provides all publicly available sources of protein-protein interaction information and performs and integrates computational predictions. String analysis pursuits to acquire a complete and global network, including direct (physical) and indirect (functional) interactions [Szklarczyk et al., 2019]. Here on this test, the gene-gene interplay was studied through string analysis. An organization of significant genes related to B lymphocytes and T lymphocytes was subjected to string analysis [Szklarczyk et al., 2019].

In a string evaluation network, the nodes constitute proteins, the edges represent the expected useful associations which can be drawn up to 7 different colored traces- those traces constitute the existence of various seven types of evidence used in predicting the institutions. The Red line shows the presence of fusion evidence, the blue line-cooccurrence evidence, the green line- neighborhood evidence, the purple line-experimental evidence, the yellow line- text mining evidence, the light blue line-database evidence, and the black line- is expression evidence [Szklarczyk et al., 2019].

String Analysis of Genes Associated with B Cells

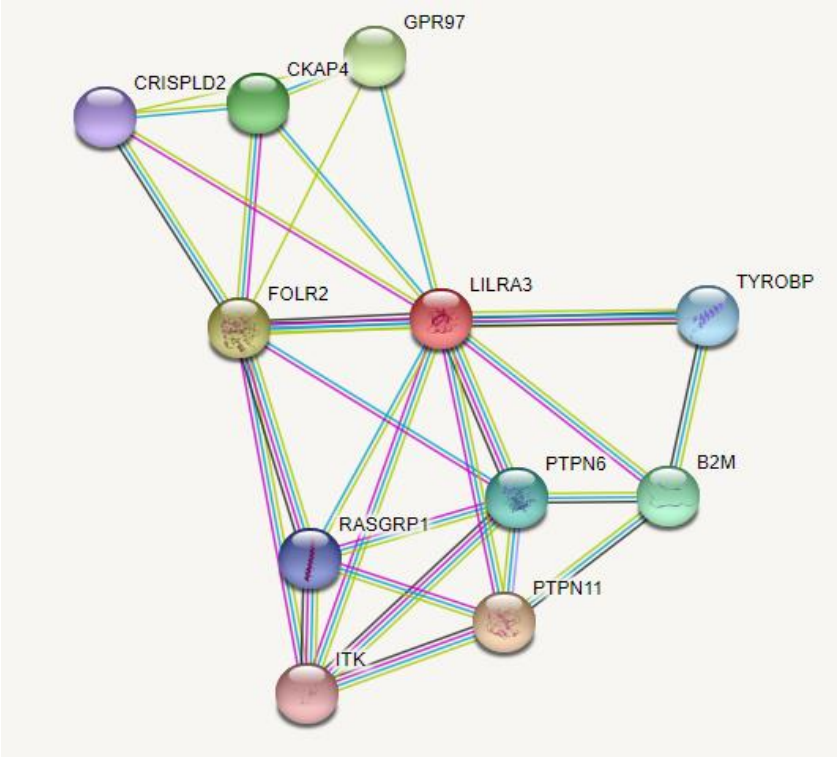

Figure 3: LILRA3 Leukocyte Immunoglobulin-like Receptor, Subfamily A, Member 3

LILRA3 is expressed on B cells and monocytes, which is related to the receptor which might suppress the feature of different LIR molecules. [Stelzer et al., 2019]

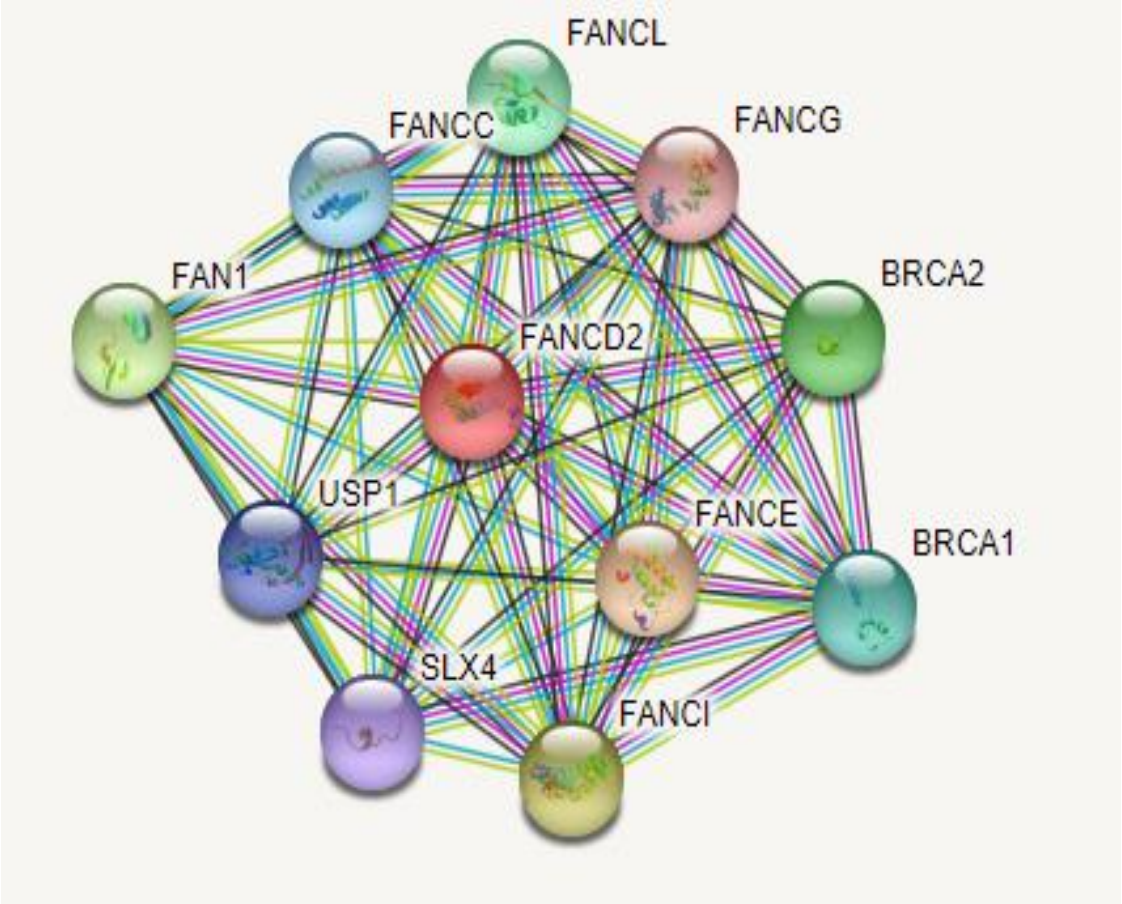

Figure 4: FANCD2 (Fanconi anemia, complementation group D2)

FANCD2 is involved in the promotion of BRCA2/FANCD1 loading onto damaged chromatin. B-cell immunoglobulin isotype switching. [Szklarczyk et al., 2019]

String Analysis of Genes Associated with Both B Cells and T Cells

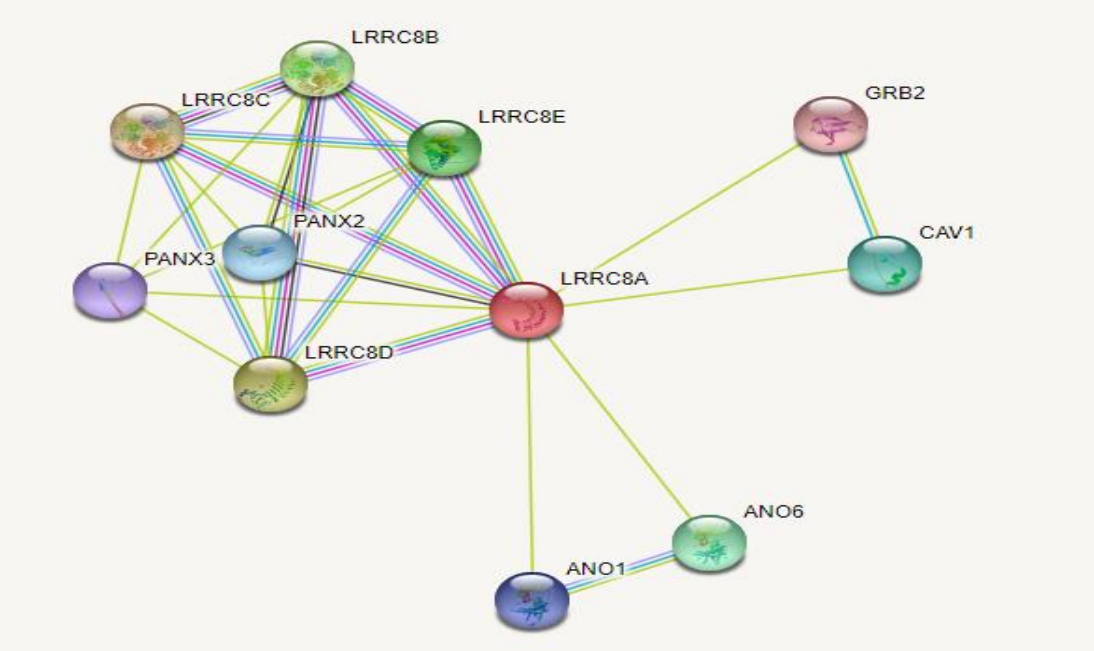

Figure 5: LRRC8A (Leucine-Rich Repeat Containing 8 VRAC Subunit A)

LRRC8A is involved in B-cell development: it is required for the pro-B cell to pre-B cell transition (PubMed:14660746). Also required for T-cell development (By similarity). [Stelzer et al., 2016]

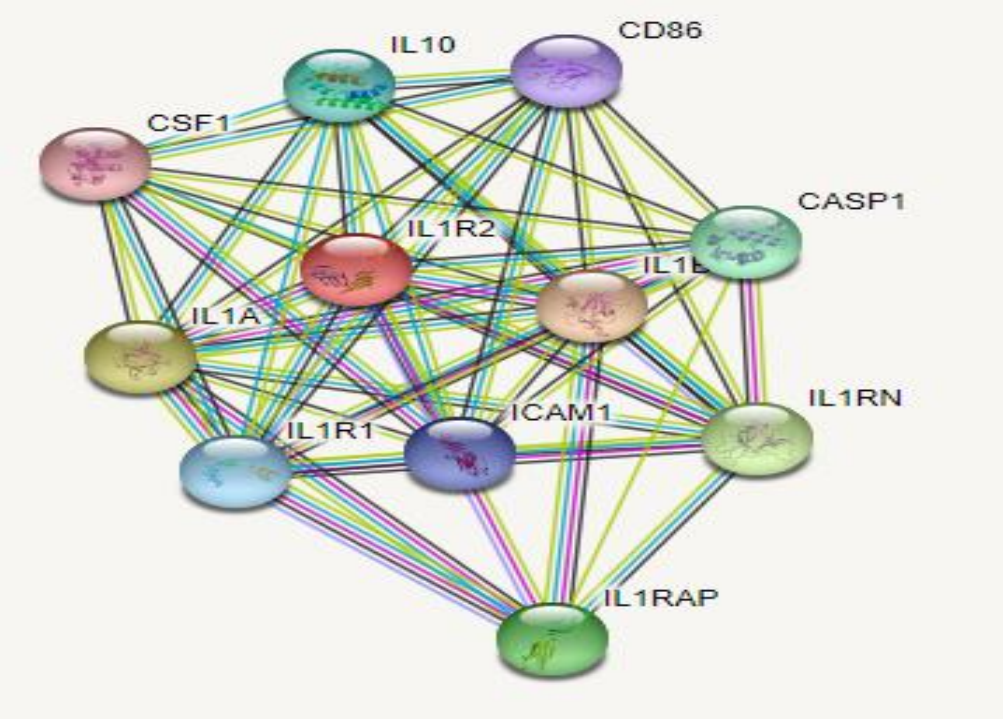

Figure 6: IL1R2 Interleukin 1 Receptor, Type 2

Expressed in B and T cells, with a transmembrane and an alternatively spliced soluble form [Szklarczyk et al., 2019].

String Analysis of Genes Associated with T Cells

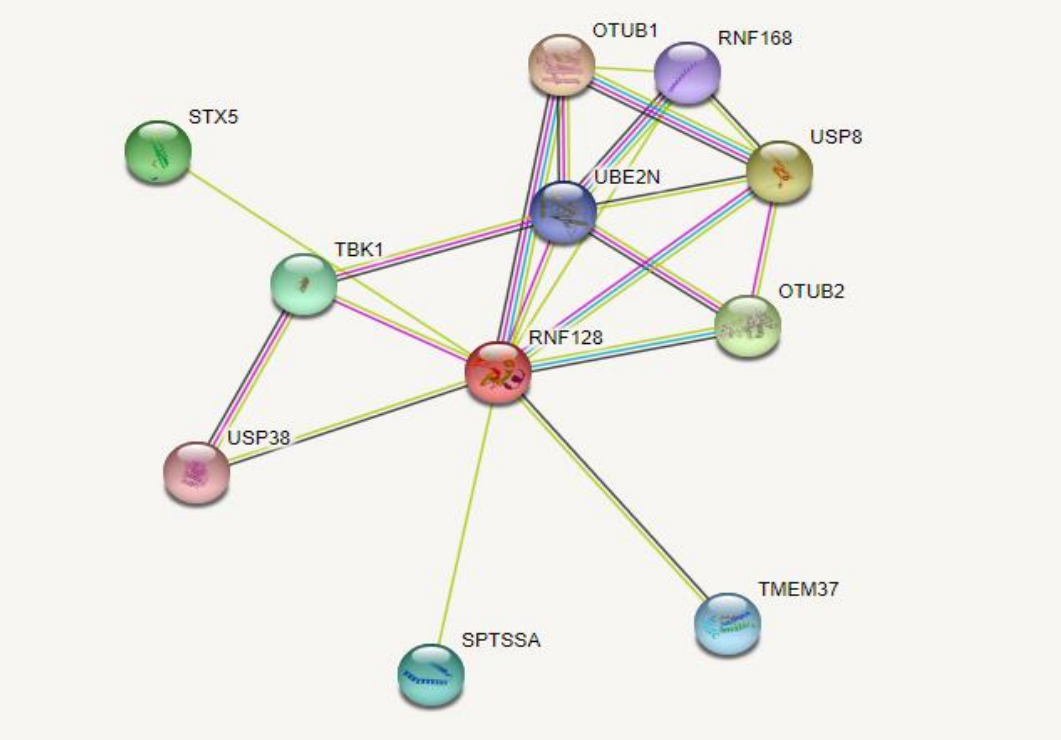

Figure 7: RNF128 (Ring Finger Protein 128)

Involves the inhibition of IL2 and IL4 transcription, thereby playing a crucial position in a long-term stable state of T-lymphocyte unresponsiveness. Antigenic stimulation is related to the blockade of interleukin manufacturing. [Stelzeret al., 2016]

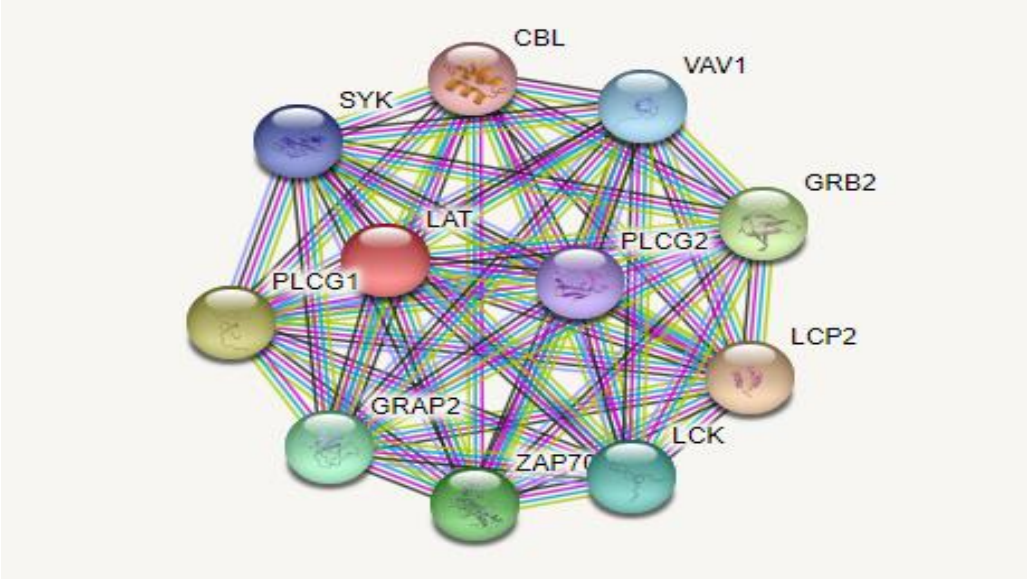

Figure 8: LAT (Linker for Activation of T Cells)

(T-cell antigen receptor)- and pre-TCR-mediated signaling, each in mature T-cells and at some stage in their improvement. Involved in FCGR3 (low-affinity immunoglobulin gamma Fc region receptor III). [Loretta et al., 2015]

Protein Structures

Proteins fold into solid three-dimensional shapes, or conformations, which are decided with the aid of using their amino acid sequence. The whole shape of a protein can be at four distinctive degrees of complexity: primary, secondary, tertiary, and quaternary structure. [Sun et al., 2004]. The promoter region controls when and in what tissue a gene is expressed. The structure of a gene's protein is precise with the aid of using the gene's coding region [Polyak et al., 2003].

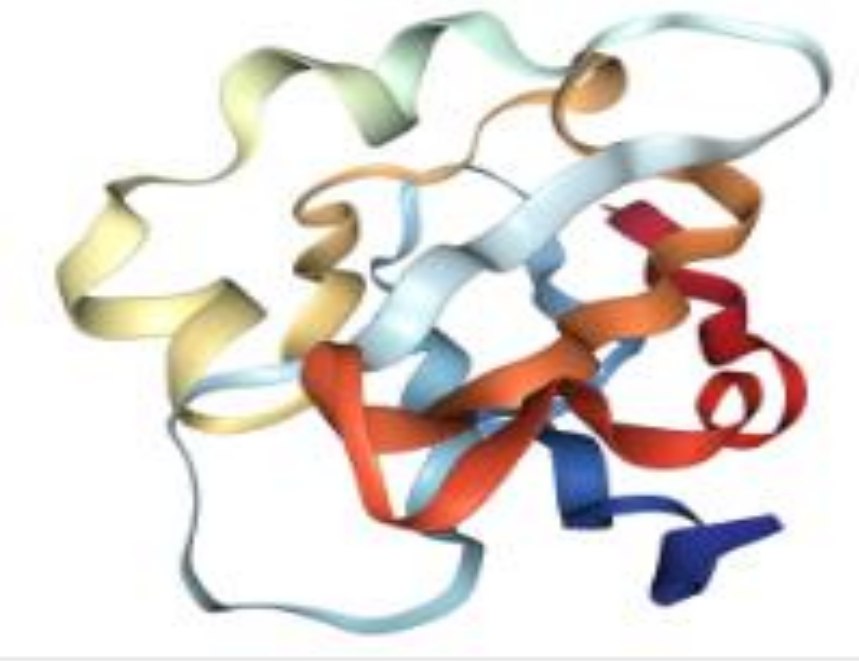

Figure 9: LILRA3 (Alpha fold, Secondary structure)

LILRA3 is involved in B-cells, and at lower levels in natural killer (NK) cells [Hornbeck et al., 2015].

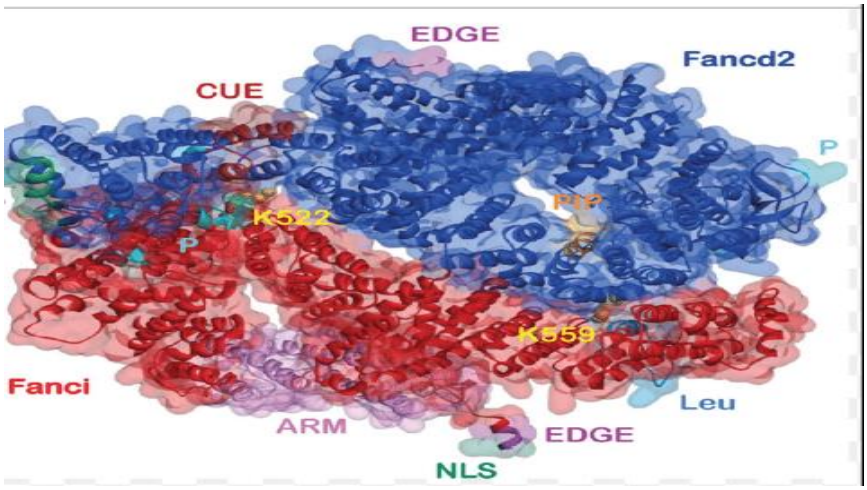

Figure 10: FANCD2 (Alpha fold, secondary structure)
FANCD2 is involved in B-cell immunoglobulin isotype switching [Hornbeck et al., 2015].

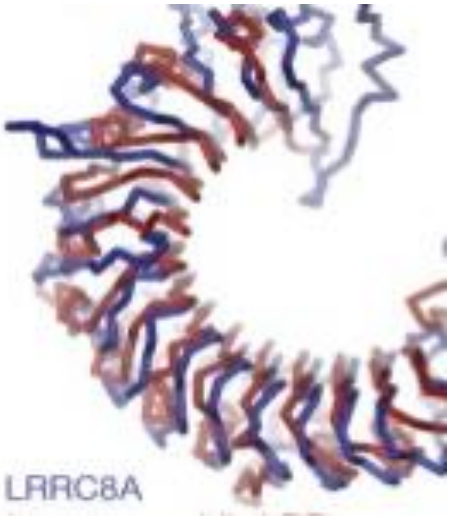

Figure 11: LRRC8A (Ribbon representation of the hexametric LRRC8A) Cα-representation of a superposition of the LRRD of LRRC8A.

LRRC8A is involved in B-cell development as it is required for the pro-B cell to pre-B cell transition. Functions in T-cell development [Hornbek et al., 2015].

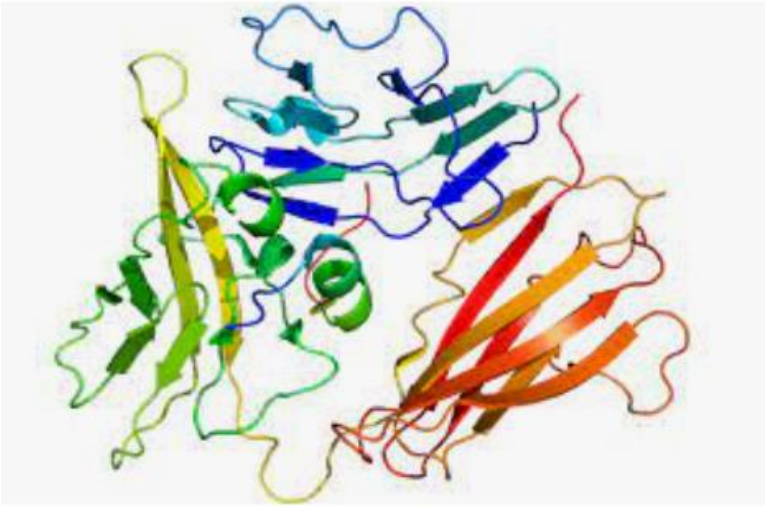

Figure 12: IL1R2 (*Orientation:* Plus, strand, Quaternary structure)

Interleukin 1 receptor, type 2, is expressed in B and T cells, with a transmembrane and an alternatively spliced soluble shape [Hornbeck et al., 2015].

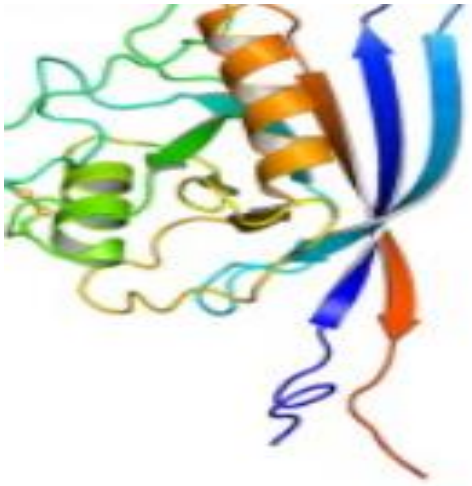

Figure 13: RNF128 (Alpha fold, secondary structure)

Plays a crucial position within a long-term stable state of T-lymphocyte unresponsiveness to antigenic stimulation that is related to the blockade of interleukin manufacturing [Hornbeck et al., 2015]

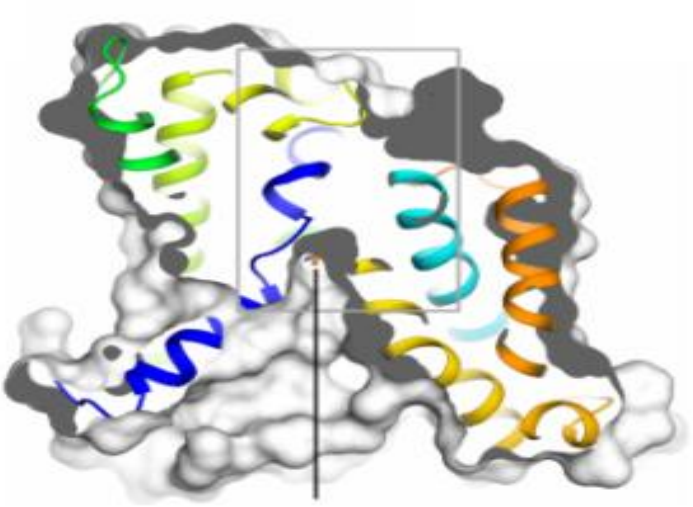

Figure 14: LAT Protein structure (Alpha fold, secondary structure)

LAT protein plays an important role during T- cells development and TCR (T-cell antigen receptor)- and pre-TCR-mediated signaling. [Hornbeck et al., 2015]

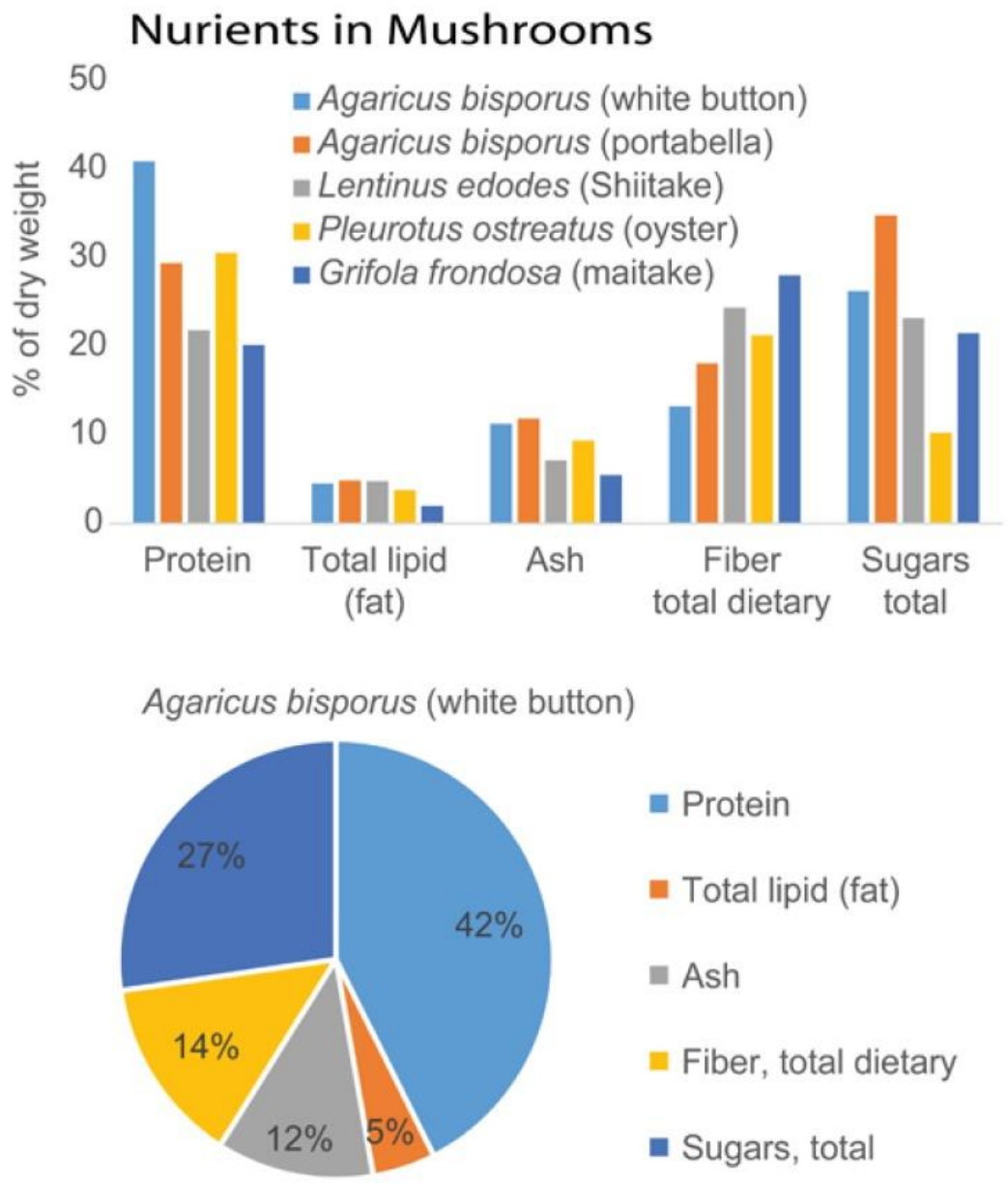

Figure 15: Nutrients in Mushroom

This figure shows the different nutrient content of five different mushrooms. The shiitake mycelia are cultured in a liquid medium used for AHCC® [Nogusa et al., 2009]

CHAPTER 5

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

It has been suggested that continuous consumption of active hexose correlated compounds linked substances may boost immunity. The outcomes of this study strongly imply that active hexose correlated compounds can assist in increased immunity. From the above-mentioned findings and observations, it can be inferred that continuous intake of Active Hexose Correlated Compounds might assist improve immune function and managing immunosuppressive side effects by encouraging leukocyte proliferation and differentiation and upregulation of leukocytes. As it is seen that genes responsible for these such as LILRA3, FANCD2, LRRC8A, IL1aAR2, RNF128, LAT, TNFSF14, VSIG4, HCST, CD1A, MLLT11, FYN, TNFSF14, CD276, TNFRSF21, are accountable for cell adhesion, proliferation, receptor signaling, triggering of T-lymphocytes and B lymphocytes and signal transduction. The cell adhesion, proliferation, receptor signaling, triggering of T- lymphocytes and B lymphocytes, and signal transduction were highly significant ($p < 0.005$). This study aims to identify elements that are produced as a result of the AHCC proliferation that facilitate the interaction between B and T cells. In this study, we explored the gene products stimulated by the AHCC exposure in T lymphocytes which play a role in B cell function using data from a previous gene array experiment to find out the effects of AHCC on the interaction between T and B lymphocytes. Hence, AHCC gives a beneficial effect on T and B cell proliferation.

A P value less than .05 was considered significant, while less than .10 but larger than .05 was regarded to have a tendency. Stat View version 4.5 was the statistical program used to conduct these studies. The outcome is expressed as mean + standard error.

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