CHAPTER I

INTRODUCTION

1.1 Statement of problems

Immune system is an important system of our body. It functions as a protective system, which plays a role to defend and eliminate pathogens. The immune system also plays a role in tumor cells protection and control balance of normal flora, which live in the body. The immune system consists of many types of cells that work together. However, leukocytes are known to be the cells which play a major role in the immune system. In the immune system, leukocytes do not work as stand-alone. They have to cooperate with other immune cells or cells of the other systems. At the present, it has become clear that cell cooperation is mediated through molecules on the cell surface (Barclay *et al.*, 1997).

The membrane proteins are components of cell membrane which are essential for cell functions. They function as enzymes, ion channels and cell receptors (Karp, 1999). Similar to other cells, the leukocytes contain many leukocyte surface molecules, which are essential for their functions. Some leukocyte surface molecules function as adhesion molecules, e.g., ICAM-1 which plays a central role in cell-cell contact-mediated immune mechanism (Boyd *et al.*, 1988). Certain molecules function as cell surface receptors such as IL-2R which plays a role in the induction of T lymphocyte proliferation (Lipkowitz *et al.*, 1984). Some molecules function as ligand such as Fas ligand, which plays a role in the initiation of apoptosis (Nagata, 1996).

CD147 is a leukocyte surface molecule, which belongs to type I integral membrane protein in the immunoglobulin superfamily (Kasinrerk *et al.*, 1992). It is a polypeptide chain of 296 amino acid with a molecular mass of 50-60 kDa (Stockinger *et al.*, 1997). It has been clustered as CD147 at the 6th International Workshop on Human Leukocyte Differentiation Antigen (HLDA workshop) (Stockinger *et al.*, 1997). The molecule also known as basigin (Miyauchi *et al.*, 1991), M6 (Kasinrerk *et al.*, 1992) and extracellular matrix metalloproteinase inducer (EMMPRIN) (Biswas *et al.*, 1995). The CD147 is broadly expressed on hemopoietic and non-hemopoietic cell lines, endothelial cells and human peripheral blood cells (Stockinger *et*

al., 1997; Kasinrerk *et al.*, 1999). This molecule is more strongly expressed on thymocytes than on mature peripheral blood T-cells (Kirsch *et al.*, 1997). In peripheral blood, CD147 expression is up regulated upon T cell activation (Kasinrerk *et al.*, 1992; 1999). CD147 is, therefore, speculated to be an essential molecule in the immune system.

The molecular function of CD147 in regulation of T cell function is not clearly understood. Association of CD147 on T cell activation has been studied. Kirsch *et al.* (1997) demonstrated that an anti-CD147 mAb, 8D6 antibody, did not have direct effect on T-cell activation or apoptosis. However, Koch *et al.* (1999) found that induction of ordered dimerization of CD147 by anti-CD147 mAb results in strong inhibition of CD3-mediated T cell activation. These controversial findings may be due to the use of antibodies that reacted to different epitopes on the CD147 molecule.

Recently, Dr. Watchara Kainrerk at the Department of Clinical Immunology, Faculty of Associated Medical Sciences, Chiang Mai University, had generated 6 monoclonal antibodies against the CD147 molecule (Kasinrerk *et al.*, 1999; un-published observations). For better understand of the molecular function of CD147 molecule, the 6 mAbs against CD147 were used to investigate the involvement of CD147 on regulation of cell proliferation and apoptosis.

1.2 Literature reviews

1.2.1 Introduction to immunology

Immunology can be defined as a study of the mechanisms, which are responsible for resistance to infection and cancer. Although these same mechanisms may occasionally also be responsible for the production of disease symptoms. The immune system is a fully integrated physiological system, which is found in all multicellular animals but is best, developed in vertebrates, and in particular mammals and birds. When a disease causing organism gets into the body, two distinct, but interrelated branches of the immune system are active, the non-specific immune response (innate immunity) and the specific immune response (adaptive immunity) (Abbas *et al.*, 2000). Both of these systems are physiological mechanisms giving the animal the ability to recognize materials as foreign to itself and to neutralize, eliminate or metabolize them.

1.2.1.1 Innate immunity

Innate immunity is the first line of host defense against microorganism. It refers to unsophisticated mechanism of host defense and antigen-nonspecific defense mechanisms that plays a more important role in primitive life forms and host uses immediately or within several hours after exposure to an antigen. Unlike adaptive immunity, innate immunity does not recognize every possible antigen. It is designed to recognize a few highly conserved structures present in many different microorganisms, which are called pathogen-associated molecular patterns such as LPS, peptidoglycan, lipotechoic acids, mannose, bacterial DNA, double-stranded RNA and glucans (Nurnberger and Brnner, 2002; Teixeira *et al.*, 2002). The principal components of innate immunity are physical and chemical barriers (epithelia cells and normal flora), cells involved in body defense (phagocytic cells, natural killer cell and cells that release inflammatory mediators) and blood protein complements (complement proteins, acute phase protein, and cytokines) (Kaiser, 2002).

1.2.1.1.1 The physical and chemical barriers

The physical and chemical barriers are the first defense mechanisms against infection. This defense mechanism is requiring anatomical barriers, mechanical removal, and bacterial antagonism by normal flora. These barriers include:

A. The skin

The skin, consisting of the epidermis and the dermis, is dry, acidic, and has a temperature lower than 37°C. These conditions are not favorable to bacterial growth. Epithelia cells produce cysteine-rich peptides that have a natural antibiotic function to kill a wide variety of bacteria and fungi (Ali *et al.*, 2001). Keratinized cells that make up the surface of the skin are continuously being sloughed off so those microbes that do colonize these cells are constantly being removed. Hair follicles and sweat glands produce lysozyme and toxic lipids that can kill any bacteria. Finally, beneath the skin surface is skin-associated lymphoid tissue (SALT) that contains cells for killing microbes and sampling antigens on the skin to start adaptive immune responses against them (Streilein, 1983; Stingl and Steiner, 1989).

B. The mucous membranes

Mucus traps microorganisms and prevents them from reaching and colonizing the mucosal epithelium. Mucus also contains lysozyme to degrade bacterial peptidoglycan, an antibody called secretory IgA that prevents microbes from attaching to mucosal cells and traps them in the mucous. Moreover, lactoferrin bind to iron and keep it from being used by microbes, and lactoperoxidase generate toxic superoxide radicals that kill microbes (Brandtzaeg, 1988a ; 1998b ; Kaiser, 2002). In addition, the mucous membrane is constantly sloughing cells to remove microbes that have attached to the mucous membranes. Beneath the mucosal membrane is mucosa-associated lymphoid tissue (MALT) that contains cells for killing microbes and sampling antigens on the mucosa to start acquired immune responses against them (Brandtzaeg, 1988a ; 1998b).

C. Bony encasements

Bony encasements, such as the skull and the thoracic cage, protect vital organs from injury and entry of microbes.

D. The cough and sneeze reflex

Coughing and sneezing removes mucus and trapped microbes.

E. Vomiting and diarrhea

These processes remove pathogens and toxins in the gastrointestinal tract.

F. The physical flushing action of body fluids

Fluids such as urine, tears, saliva, perspiration, and blood from injured blood vessels also flush microbes from the body.

G. Normal floras

The normal floras keep potentially harmful opportunistic pathogens in check and also inhibit the colonization of pathogens by used metabolic products (fatty acids, bacteriocins, *etc.*) that inhibit the growth of many pathogens, adhering to target host cells thus covering them and

preventing pathogens from colonizing, depleting nutrients essential for the growth of pathogens and nonspecifically stimulating the immune system (Kaiser, 2002).

1.2.1.1.2 Cells involved in body defense

A. Defense cells in the blood

All leukocytes in the blood are critical to body defense. There are normally between 5,000-10,000 leukocytes per cubic millimeter (mm^3) of blood and these can be divided into five major types including neutrophils, basophils, eosinophils, monocytes, and lymphocytes. The defense mechanisms are following through:

Neutrophils

Neutrophils are important phagocytes, which contain granules with various agents for killing microbes. These granules include lysozyme, lactoferrin, acid hydrolase, and myeloperoxidase (O'Donnell and Andersen, 1982). These agents not only kill microbes intracellularly during phagocytosis but are also released extracellularly. Once released, these agents affect not only microbes but also surrounding cells and tissue (Parslow *et al.*, 2001; Kaiser, 2002).

Eosinophils

Eosinophils contain granules with destructive enzymes for killing infectious organisms. These enzymes include acid phosphatase, peroxidases and proteinases. These cells are capable of phagocytosis but primarily they release their contents into the surrounding environment to kill extracellular microbes such as fungi, protozoa, and parasitic worms that are too big to be consumed by phagocytosis. Finally, the eosinophils can secrete leukotrienes and prostaglandins promote inflammation by causing vasodilatation and increasing capillary permeability.

Basophils

Basophils contain granules, which release histamine, leukotrienes, and prostaglandins. These chemicals promote inflammation by causing vasodilation, increasing capillary permeability and increasing mucous production.

Monocytes

Monocytes are important phagocytes that differentiate into macrophages when they leave the blood and enter the tissue. Macrophages and dendritic cells are very important in phagocytosis and aid in the adaptive immune responses. They produce a variety of cytokines that plays numerous roles in body defense.

Natural killer cells (NK cells)

NK cells are lymphocytes that lack B-cell receptors and T-cell receptors. They kill cells bound by antibody through antibody-dependent cell cytotoxicity (ADCC) or cells that lack MHC-I molecules on their surface (Parslow *et al.*, 2001).

B. Defense cells in the tissue

The defense mechanisms in the tissue are as follow:

Macrophages

When monocytes leave blood circulation and enter tissue, they become activated and differentiated into macrophages. Macrophages kill microorganism by phagocytosis. These cells can help adaptive immune responses by processing antigens so they can be recognized by T-lymphocytes during the adaptive immune responses. Macrophages also secrete proteins called cytokines that play a variety of roles in nonspecific body defense. These cytokines induce tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8) (Abbas *et al.*, 2000; Kaiser, 2002).

Dendritic cells

Dendritic cells are considered to be the most potent antigen presenting cells (APCs) in the body that found under the skin and mucous membranes. Dendritic cells kill microorganisms by taking up microorganisms through endocytosis and pinocytosis. They initiate adaptive immune responses by presenting processed antigens to T-lymphocytes (Palucka and Banchereau, 2002). Finally, dendritic cells also produce the same cytokines as the macrophages.

Mast cells

Mast cells are found throughout the connective tissue of the skin and mucous membranes and they carry out the same functions as basophils. They can release histamine, leukotrienes and prostaglandins (Kaiser, 2002). These chemicals promote inflammation by causing vasodilation increasing capillary permeability and increasing mucous production (Hart, 2001). Mast cells usually the first cells to initiate inflammatory response.

B-1 cells

B-1 cells found in the peritoneal cavity, whose antigen receptors are immunoglobulin molecules that are produced by somatic gene recombination but have limited diversity. B-1 cells are specific for polysaccharide and lipid antigens present on diverse bacteria. These cells can produce IgM antibodies (natural antibodies) against bacteria that are commonly found in the environment (Abbas *et al.,* 2000).

1.2.1.1.3 Blood protein complements

The defense mechanisms via blood protein complements are as follow:

A. The complement system

The complement system refers to a series of proteins circulating in the blood and bathing surrounding tissues. In normal situation, these protein are in inactive forms, but in response to the recognition of molecular components of microorganism, they become activated. The proteins in complement system are working in a cascade because the binding of one protein will promote the binding of the next protein in the cascade. Initiation of the complement cascade can occur via three pathways i.e., classical complement pathway, lectin pathway and alternative complement pathway (Song *et al.*, 2000; Kaiser, 2002). The final results and defense benefits from each pathway are the same in that complements trigger inflammation, chemotactically attract phagocytes to the infection site, promote the attachment of antigens to phagocytes opsonization, cause lysis of gram-negative bacteria and human cells displaying foreign epitopes and remove harmful immune complexes from the body (Song *et al.*, 2000).

B. Acute phase proteins

The acute phase proteins are certain blood proteins, which increased in their production when the body exposes to microorganism during acute illness. The proinflammatory cytokines released from activated macrophages and other leukocytes stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins. The members of acute phase proteins include

1. C-reactive protein (CRP); binds to phospholipids in microbial membranes. The functions of CRP this is to opsonize microorganisms to phagocytes as well as to activate the classical pathway of the complements (Abbas *et al.*, 2000).
2. Mannose-binding lectin (MBL); binds to mannose sugars found in many bacteria and fungi but not in mammalian cells. MBL functions as opsonins and also activates the lectin pathway (Abbas *et al.*, 2000).

C. Cytokines

Cytokine is the name given to a wide variety of intercellular regulatory proteins produced by many different cells in the body that ultimately controls every aspect of body defense. Cytokines play role in activation and inactivation of phagocytes and other immune defense cells and promote or inhibit a variety of nonspecific body defenses (Abbus *et al.*, 2000; Kaiser, 2002). Examples of cytokines are interleukins (IL), tumor necrosis factors (TNF), colony-stimulating factors (CSF), chemokines, and interferons (IFN).

1.2.1.2 Adaptive immunity

Adaptive immunity is the next line of host defense for microorganism after the innate immunity. It refers to as antigen-specific defense mechanisms in which the process takes several days to cause microbial protection and elimination of a specific antigen. This is the immunity that one develops throughout life. There are two major branches of the adaptive immune responses; humoral immunity and cell-mediated immunity.

1.2.1.2.1 Humoral immunity (HMI)

Humoral immunity refers to the production of antibody molecules in response to an antigenic stimulus. These antibody molecules circulate in the blood and enter the tissue via

inflammation. Eeffector functions of antibodies are neutralization and elimination of infected microbes and microbial toxins.

Antibodies or immunoglobulins are specific glycoproteins produced by plasma cells, a terminally differentiated B lymphocytes in the lymphoid organs and bone marrow. Antibodies, however, perform their effector functions distantly from their production sites. The first exposure to an antigen leads to the activation of naïve B-lymphocytes. As a consequence, B-lymphocytes differentiate into either antibody-producing cells or memory cells. The antibodies may be derived from long-lived antibody-producing cells or memory B cells that migrate to the bone marrow and persist there leading to secondary immune response.

A. The structure of antibodies (Kaiser, 2002)

The human antibodies are composed of 5 classes of immunoglubulins namely, IgG, IgM, IgA, IgD, and IgE. These "Y"-shaped macromolecules, each called monomers which is composed of four glycoprotein chains. There are two identical heavy chains with high molecular weight that varies with the class of antibody. In addition, there are two identical light chains with lower molecular weight. There are two varieties of light chains: kappa and gamma. The four glycoprotein chains are connected to one another by disulfide (S-S) bonds and noncovalent bonds.

Additional S-S bonds fold the individual glycoprotein chains into a number of distinct globular domains. The area where the top of the "Y" joins the bottom is called the hinge. This area is flexible to enable the antibody to bind to pairs of epitopes at various distances apart on an antigen.

The two tips of the "Y" monomer are referred to as the Fab portions of the antibody that provide specificity for binding an epitope on an antigen. The antigen binding site is large enough to hold an epitope of about 5-7 amino acids or 3-4 sugar residues.

The bottom part of the "Y", the C terminal region of each glycoprotein chain, is called the Fc portion. The Fc portion, as well as one domain of both the heavy and light chain of the Fab region has a constant amino acid sequence that defines the class and subclass of each antibody. The Fc portion is responsible for the biological activity of the antibody.

Because the effector functions of antibodies are mediated by the heavy chain constant regions of immunoglobulin (Ig) molecules, different Ig heavy chain isotypes serve distinct effector functions. The effector functions of antibody isotypes are listed in Table 1.1.

Table 1.1 The effector functions of antibody isotypes.

Antibody isotype	**Isotype-specific effector functions**
IgG	Activation of the classical complement pathway
	Binding to macrophages and neutrophils for enhanced phagocytosis
	Binding to NK cells for antibody-dependent cytotoxicity (ADCC)
	Neonatal immunity: Transfer of maternal antibody across the placenta and gut
	Feedback inhibition of B cell activation
IgM	Activation of the classical complement pathway
	Antigen receptor of naïve B lymphocytes
IgA	Activation of the alternative complement pathway
	Mucosal immunity: Secretion of IgA in to the lumens of the gastrointestinal and respiratory tracts
	Neonatal immunity: Transfer of maternal antibody from breast milk
IgE	Response to parasitic worms and often in response to allergens
	Antibody-dependent cell-mediated cytotoxicity involving eosinophils
	Mast cell degranulation
IgD	Play a role in eliminating B-lymphocytes that generate self-reactive autoantibodies

B. The mechanism for humoral immunity

At the first step of HMI, foreign antigen encounters B-lymphocytes, which have surface immunoglobulin (BCR) specific to the antigen. The binding of specific antigen with BCR on B-lymphocytes leads to two responsive ways of B-lymphocyte activation. First, once the binds to BCR, the antigen is then endocytosis and after endosome-lysosome fusion into a series of peptide epitopes. (Parslow *et al.*, 2001; Abbas *et al.*, 2000; Kaiser, 2002). These peptides are eventually placed in grooves of MHC-II molecules, which are then transported to the surface of the B-lymphocyte. Then, TCR on $CD4^+$ T lymphocytes, in cooperation with the CD4 molecules and co-stimulatory molecules, recognizes this foreign peptides and the interaction leads to T cell activation. Subsequently, this activation induces activated T cells to synthesize CD40L and cytokines such as IL-4, IL-5, IL-6 and IL-10. There molecules provide co-activation signals for B cell activation (Parslow *et al.*, 2001).

The second responsive ways of B-lymphocyte activation is through cross-linking of BCR. The immunoglobulin cross-linking by antigen can induce a signal through the BCR alone or the transmission can be enhanced when the antigen is associated with other immunologically relevant ligands such as the C3d fragment of complement (Parslow *et al.*, 2001). The generated signals can lead to activate B-lymphocytes proliferation and differentiation to plasma cells.

After activation, B-lymphocytes proliferate into many clones of identical BCR. B-lymphocytes proliferation and differentiation depend strictly on T_H2. Antibodies secreted from plasma cells are capable of reacting with B cell epitope of the original initiating antigen causing it to be destroyed or neutralized (Parslow *et al.*, 2001). Notably, some of the activated B-lymphocytes, which do not differentiate into plasma cells, instead revert to the resting state to become memory B-lymphocytes that have a long life. These memory B cells circulate throughout the body waiting to encounter with the same antigen once it enters the body. Memory B-lymphocytes are capable of inducing anamnestic response, a heightened secondary response to the antigen (Parslow *et al.*, 2001; Kaiser, 2002).

C. The response of humoral immunity

Neutralization of microbes and microbial toxins.

In viral neutralization, antibodies are made against viral capsid or envelope to prevent virus from adsorbing to host cells as well as preventing viral re-infection. For bacteria, antibodies are made against pili, capsules, and adhesins to prevent bacteria from adhering to and colonizing host cells. If antibodies are made against flagella of motile bacteria, flagella or cilia of motile protozoa, the bound antibodies will arrest their movement and block their ability to spread. For toxin neutralization, antitoxin antibodies (mainly IgG) are made against exotoxins of bacteria. The binding of antibody to exotoxin will prevent exotoxin interaction with host target cells and thus neutralize the exotoxin (Abbas *et al.,* 2000; Kaiser, 2002).

Antibody-mediated opsonization and phagocytosis.

Antibodies of IgG isotype which bind to a surface antigen of the microbes will promote phagocytosis through the binding to Fc receptor on phagocytes. Alternatively, IgG and IgM can activate the classical complement pathway resulting in the generation of C3b or C4b that can also and promote phagocytosis (Abbas *et al.,* 2000; Kaiser, 2002).

Antibody-dependent cellular cytotoxicity (ADCC) by NK cells

Once IgG binds to a surface antigen of microbes, it promotes microbial cell lysis by NK cells through antibody-dependent cellular cytotoxicity or ADCC (Abbas *et al.,* 2000). The NK cells possess receptors on their surface for the Fc portion of antibodies. When antibodies are made against foreign cell, the NK cells then bind to the Fc portion of the antibody and lyse foreign cell by inducing membrane lesions with pore-forming perforins or inducing apoptosis of target cells through the action of cytotoxic granzymes (Abbas *et al.,* 2000; Kaiser, 2002).

1.2.1.2.2 Cell-mediated immunity (CMI)

Cell-mediated immunity is the results of effector function of T lymphocytes. It is an immune response that does not involve antibodies but rather involves antigen-specific cytotoxic T-lymphocytes (CTLs). The CTLs are able to lyse target cells, which display epitopes of foreign antigen on their surface in context of MHC-I molecules . In addition to target cell lysis, CMI also

contributes to production of cytokines that influence the function of other immune cells involved in adaptive immune response and innate immune response.

Primarily, CMI not only is the most effective system in removing virus-infected cells but also participates in the defense against fungi, protozoa, cancerous cells, and intracellular bacteria. It also plays a major role in transplant rejection.

A. The mechanism of cell-mediated immunity

At the very first step of CMI, foreign antigen encounters the antigen-presenting cells (APCs) such as macrophage, dendritic cell, B-cell or cells which can express MHC class I or/and class II molecule (Abbas *et al.,* 2000; Kaiser, 2002). For the endogenous antigens from intracellular pathogen such as intracellular bacterias and viruses, these antigens are degraded by proteasomes. Protein antigens are broken down into a series of peptide epitopes. These peptides eventually lie in the grooves of MHC-I molecules and are then transported to the surface of APCs. In the case of exogenous antigens from extracellular pathogen such as bacteria, these antigens are endocytosed and degraded by lysozyme after andosome-lysosome fusion. The processed peptides eventually bind to MHC-II molecules and are then transported to the surface of APCs

Subsequently, T cell receptors (TCRs) on naive $CD4^+$ T recognize peptides from exogenous antigens bound to MHC-II molecules on the surface of APCs. The interaction is the first signal, which is necessary for initiation of $CD4^+$ T cell activation (Parslow *et al.,* 2001; Kaiser, 2002). Additionally, the second signal is required in order to prevent T cell angry. Interaction of co-stimulatory molecules like CD28 on T cell to B7 molecules on APC is essential for the activation of naïve $CD4^+$ T cells to T_H0 (Medzhitov and Janeway, 1997; Roitt, 1997).

The T_H0 produce both interleukin-2 (IL-2) and its corresponding receptor, IL-2 receptor. Then, the secreted IL-2 binds to IL-2 receptor on the T_H0 cell enabling it to proliferate into a large number of T_H0 clones. Finally, these clones undergo differentiation into T_H1 or T_H2 cells when expose to different cytokines. IL-12 is a major inducer of T_H1 cells while IL-4 is a major inducer of T_H2 cells. Additionally, the same cytokines may function as growth factor to expand the respective subsets (Abbas *et al.,* 2000; Kaiser, 2002).

Regulatory $CD4^+$ T cells can be divided into 2 different types based on the cytokines they produce. The T_H1 cells recognize antigens presented by macrophages and have primarily function to activate and promote CMI by producing cytokines such as IL-2, IFN-γ, and TNF-β. T_H2-lymphocytes recognize antigens presented by macrophages and B-lymphocytes. They produce cytokines such as IL-4, IL-5, IL-9, IL-10, and IL-13, which promote antibody production (Abbas *et al.*, 2000; Kaiser, 2002).

Similar to $CD4^+$ T cells, the T cell receptor (TCR) on $CD8^+$ T cells is capable of recognizing foreign peptide from endogenous antigen bound to MHC-I molecule on the surface of APCs and convey the first signal that is necessary for activation of the $CD8^+$ T cells (Parslow *et al.*, 2001; Kaiser, 2002). Then, the co-stimulatory molecules such as B7 molecules on the APC and CD28 on $CD8^+$ T cells interact and give the second signal for activation of the $CD8^+$ T cells (Medzhitov and Janeway, 1997; Roitt, 1997). These two stimulatory signals activate the proliferation of $CD8^+$ T cells to eventually differentiate into many CTLs, which can bind and kill the virus-infected cell by inducing apoptosis through perforin, granzymes and Fas ligand (Abbas *et al.*, 2000; Kaiser, 2002).

Finally, some of the activated T-lymphocytes differentiate into circulating $CD4^+$-memory T cells, $CD8^+$-memory T cells, and $CD8^+$-suppressor T cells. Circulating $CD4^+$-memory T cells and $CD8^+$-memory T cells allow for a more rapid and greater production of CTLs and cytokines upon subsequent exposure to the same antigen (Kaiser, 2002).

The $CD8^+$-suppressor T cells help to turn off both HMI and CMI by destroying activated B-lymphocytes and T-lymphocytes through the FasL/Fas interaction in apoptosis with a programmed cell suicide (Kaiser, 2002).

B. The response of cell-mediated immunity

CMI responds to pathogens in 3 ways. The first way is to activate macrophages and NK cells via the cytokines such as IL-2 and IFN-γ, which are enable then to destroy pathogens (Abbas *et al.*, 2000). Secondly, antigen-specific CTLs are activated to lyse the infected cells via the release of intracellular granules containing perforins and proteolytic enzymes, or via FasL/Fas interactions (Nagata, 1996; Abbas *et al.*, 2000; Parslow *et al.*, 2001). Thirdly, cells of CMI are stimulated to secrete a variety of cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10,

Il-12, interferon (IFN), tumor necrosis factors (TNF) and colony-stimulating factors (CSF) that cast influence on the function of other cells involved in adaptive immune response and innate immune response (Abbas *et al.*, 2000; Belardelli and Ferrantini, 2002).

1.2.2 Cell cooperation

Every system or organ is composed of many cells. Because of cell do not live in isolation, every cell needs to cooperate to one another for function and survival.

1.2.2.1 Extracellular signaling molecules in cell cooperation

A. Cytokines

Cytokines are low molecular weight, soluble proteins that function as chemical messengers for regulating the immune system. They are produced by the cells of innate and adaptive immunity, especially by T helper lymphocytes (Kaiser, 2002). The activation of cytokine-producing cells trigger them to synthesize and secrete their cytokines. The cytokines, in turn, are then able to bind to specific cytokine receptors on other cells of the immune system and influence their activities in some manners. The nomenclature of cytokines is often based on their cellular sources. Cytokines that are produced by mononuclear phagocytes are sometimes called monokines, and those produced by lymphocytes are commonly called lymphokines (Abbas *et al.*, 2000). Because many cytokines are made by leukocytes and act on other leukocytes, they are also called interleukins (IL). Examples of cytokines are IL-4, IL-5 and IL-10 which are produced by CD4+ T lymphocytes (T_H2).

B. Chemokines

Chemokines are a large family of structurally homologous cytokines that mobilize white blood cells (WBCs) to sites of inflammation (Abbas *et al.*, 2000; Kaiser, 2002). They pull the WBCs out of the blood stream and chemotactically attract them to the inflammatory site, trigger some WBCs to release their killing agents for extracellular killing, and induce some WBCs to ingest the remains of damaged tissue.

C. Interferons (IFN)

Interferons modulate the activity of virtually every component of the immune system. Type I IFNs is composed of distinct groups of proteins called IFN-α which has more than 20 types, IFN-β, interferon o, and interferon τ (Abbas, 2000). There is only one type II IFN, i.e., IFN-γ. Type I IFNs, which can be produced by virtually any virus-infected cell is able to induce viral resistance in neighboring cells. Type II IFN is produced by activated T-lymphocytes as part of an immune response and functions mainly to promote activity of the components of cell-mediated immune system such as CTLs, macrophages, and NK cells (Kaiser, 2002).

D. Tumor necrosis factors (TNF)

TNF is the principle mediator of the acute inflammatory response to infectious microbes and is responsible for many of the systemic complications of severe infection (Abbas *et al.*, 2002). TNF-α is produced by monocytes/macrophages, T_H1 cells, and other cells. TNF-β is produced by T_H1 cells and T8-lymphocytes.

E. Hormone

Hormone is a chemical or protein product. It can be separated into three broad categories (Lodish, 2000). The first category is composed of small lipophilic molecules that diffuse across the plasma membrane and interact with intracellular receptors. The second category includes hydrophilic molecules that bind to cell-surface receptors and lipophilic that bind to cell-surface receptors are classified as the third group.

F. Membrane-bound signal molecules

The membrane-bound signal molecules are membrane proteins on cell surface that function as ligand. They are synthesized within the cell via mRNA, ER membrane, and presented on the cell plasma membrane. The synthesis depends by three ways. First, it is constitutively synthesized and it function when combined with other protein. Example of this molecule is MHC molecule, which function as ligand when peptide antigen is inserted in to this molecule. Second, also constitutively synthesized and is ready to function. Examples of these molecules are CD28, B7, ICAM-1, which function as co-stimulatory molecules. Third, it is induced to synthesize

dependable on signaling requirement. Examples of the third type are Fas ligand, which is produced by CTL and CD40L, which is produced by $CD4^+$ helper T lymphocytes (Lowin *et al.*, 1994; Armitage *et al.*, 1993).

1.2.2.2 T cell activation and signal transduction

1.2.2.2.1 MHC-TCR complex mediated-T cell activation

T lymphocytes, the antigen-specific cells of the immune system, are generally quiescent and requite antigen stimulation to progress from naïve cells. In order to activate T cells, naturally, the signaling from the T cell antigen receptor (TCR) complex through TCR and co-stimulatory receptors are mainly pathway in requirement of T cell activation (Weiss and Littman, 1994). Normally, the complete TCR is a complex of eight polypeptide chains, two of which are the highly variable α and β chain, which provide the single antigen-binding site. The invariant accessory chains are CD3γ, CD3δ and CD3ε, which make up the CD3 complex along with a largely intercytoplasmic homodimer of ζ chains (Oettgen and Terhorst, 1987; Janeway *et al.*, 1999).

When TCR become clustered on binding MHC:peptide complexes on the surface of APC, activation of receptor-associated kinases such as Fyn leads to phosphorylation of the CD3γ, δ and ε ITAMs as well as those on the ζ chains (Kane *et al.*, 2000; Oettgen and Terhorst, 1987; Iwashima *et al.*, 1994). Then, the tyrosine kinase ZAP-70 binds to the phosphorylated ITAMs of the ζ chains but is not activated until binding of the co-receptor (CD4 or CD8) to the MHC molecule on the APC binding the kinase Lck into the complex (Janeway *et al.*, 1999). Lck then phosphorylates and activates ZAP-70. After ZAP-70 activation, it leads the important signaling pathways, which phosphorylates tyrosines on various adapter molecules and become docking sites for cellular enzymes such as phospholipiase C-γ (PLC-γ) and Ras GTP/GDP exchange factor (Abbas *et al.*, 2000; Weiss and Littman, 1994).

The activated and Ras GTP/GDP exchange factor (e.g. Sos) converts Ras-GDP to Ras-GTP and it then activates cascade of MAP kinase (Abbas *et al.*, 2000; Weiss and Littman, 1994). This culminates in the activation of Fos and hence of the AP-1 transcription factors (Janeway *et al.*, 1999). Moreover, PLC-γ then cleaves PIP_2 into DAG and IP_3 (Weiss and Littman, 1994). DAG activates protein kinase C (PKC) and leads to activation of the transcription factor NFκB

whereas IP_3 increases intracellular free Ca^{2+} concentration and then leads to activation of cytoplasmic phosphatase and calcineurin, which enables the transcription factor NFAT to translocate from the cytoplasm to the nucleus (Janeway *et al.,* 1999; Weiss and Littman, 1994). Full transcriptional activity of NFAT also requires a member of the AP-1 family of transcription factors; these are dimers of members of the Fos and Jun families of transcription regulators (Janeway *et al.,* 1999). Finally, in the cooperated with helper signal from co-stimulatory molecules such as CD28, the transcription factor NFκB, NFAT, Fos/Jun and AP-1 act on the chromosomes, initiating new gene transcription that results in the activation of T cells. T cell activation and signaling is summarized in Figure 1.1.

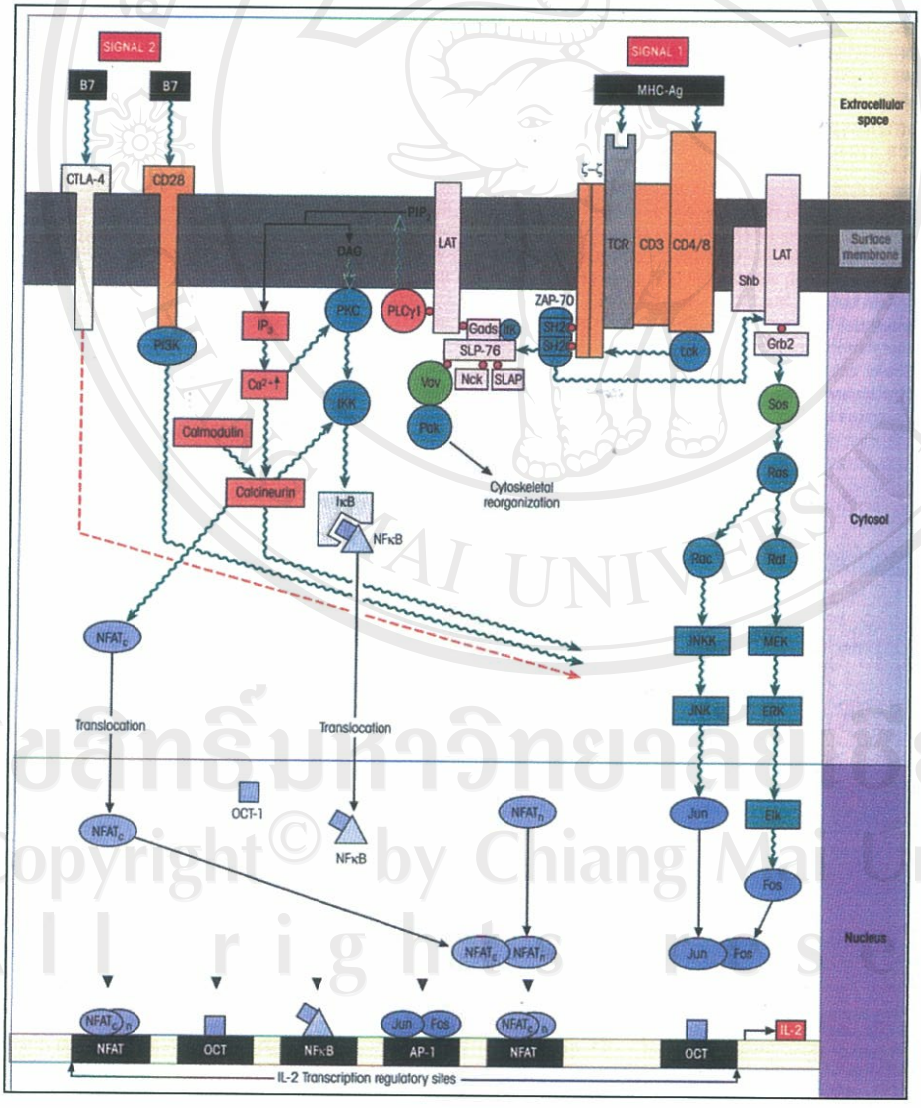

Figure 1.1 T cell signaling leads to activation (Roitt and Delves, 2001).

1.2.2.2.2 CD3-mediated T cell activation

The TCR on T cells is highest antigen-specific receptor. The down stream signal transduction and T cell activation will not occur if peptide antigen on MHC:peptide complexes is not specific to the TCR. Therefore, in order to imitate signal transduction through TCR complex *in vitro*, anti-CD3 antibody is utilization in stead of specific antigen. Interaction of anti-CD3 antibody to TCR-CD3 complex induces signal transduction through the TCR-CD3 complex. OKT3, a mAb specific for the CD3ε portion of the TCR complex, has been used in many experiments and clinically treatment for more than twenty years ago (Cosimi *et al.,* 1981). This monoclonal antibody directly induces signal transduction of TCR complex through CD3 molecule (Krutmann *et al.,* 1990; Smith and Bluestone, 1997).

The T cell signaling pathway induced by anti-CD3 mAb is almost the same as those occurred in MHC:peptide complexes induced T cell activation. Anti-CD3 mAb induces T cell activation by cross-linked CD3 molecules with specific it (Lamers *et al.,* 1992; Smith and Bluestone, 1997). In CD3 induced signaling pathway, after anti-CD3 mAbs bind and cross-link CD3 molecules, phosphorylation of the CD3γ, δ, ε and the ζ chains is initiated (Kane *et al.,* 2000; Oettgen and Terhorst, 1987; Weiss and Littman, 1994). The Phosphorylation involves the actions of the Src kinase Lck, and potentially Fyn, and leads to the association of ZAP-70 to the TCR complex and subsequent ZAP-70 phosphorylation (Smith and Bluestone, 1997). These signaling events induce a variety of signaling pathways which are the same as MHC-TCR complex mediated-T cell activation pathways, including PLC-γ1 signaling which activates Ca^{2+} flux and PKC activation, and Ras signaling which activates MAP kinase (Weiss and Littman, 1994). These signaling cascades culminate in the activation of transcription factors such as NF-AT, AP-1 and NFκB, which stimulate the expression of genes such as IL-4 and IL-2 and activate T cells (Weiss and Littman, 1994; Smith and Bluestone, 1997).

However, if anti-CD3 antibody does not cross-link CD3 molecules such as in the case of using soluble anti-CD3 mAbs. This results in partial phosphorylation of the ξ chains and TCR association of un-phosphorylated ZAP-70, possibly reflecting insufficient Lck recruitment (Smith and Bluestone, 1997). Downstream of the TCR complex signaling, PLC-γ1 and MAP kinase activation, are impaired and results as no T cell activation (Figure 1.2).

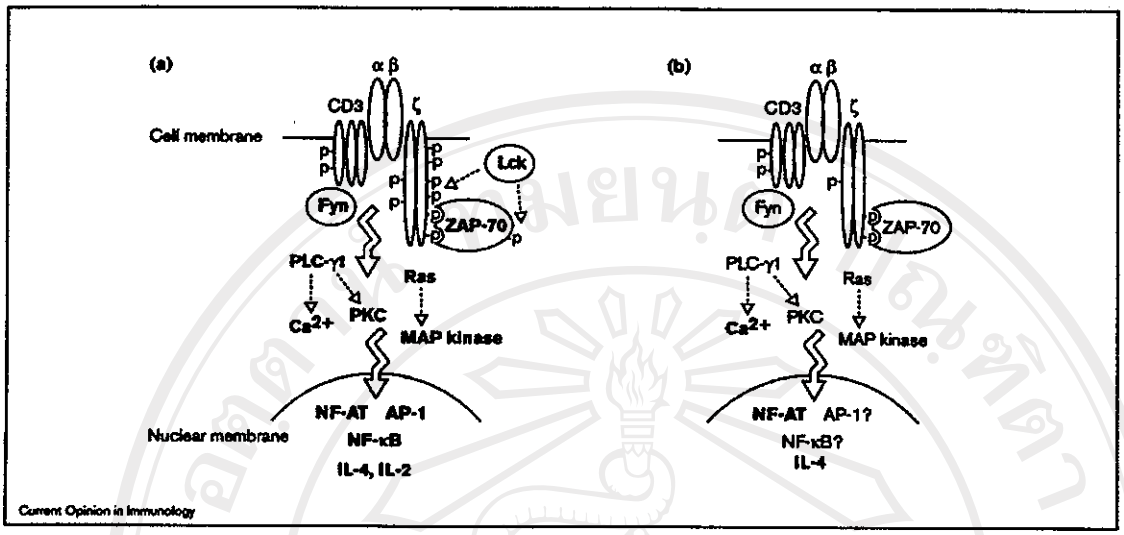

Figure 1.2 Insufficient TCR aggregation results in the generation of a partial signal. (a) The αβTCR stimulated with cross-linked anti-CD3 mAbs. (b) αβTCR stimulated with soluble anti-CD3 mAbs. Dotted arrows indicate signal transduction pathways with intermediary steps between the specified molecules. Wavy arrows indicate transmission of these cytoplasmic signaling cascades into the nucleus (Smith and Bluestone, 1997).

1.2.3 Apoptosis

Apoptosis refers to the morphologic features of programmed cell death, which is characterized by cell shrinkage, nuclear condensation, membrane blebbing, fragmentation into membrane bound apoptotic bodies, and membrane changes that eventually lead to phagocytosis of the affected cells (Saikumar *et al.*, 1999). It is important biological roles in the homeostasis of cell populations, development and pathogenesis. Excessive or insufficient apoptosis contributes to the pathogenesis of a wide variety of diseases related to ischemia, autoimmunity, and viral infections, and is involved in the growth and regression of tumors (Everett and McFadden, 1999; Saikumar *et al.*, 1999).

Apoptosis was first characterized during genetic studies on the nematode worm *Caenorhabditis elegans* (Ellia and Horvitz, 1986). However, in mammalian cells, the programmed cell death is far more complex than in the worm *C. elegans*, but generally follow the same pattern. The programmed cell death in mammalian cells is tightly regulated and is mainly

orchestrated by the activation of the aspartate-specific cysteine protease (caspase) cascade (Zimmermann *et al.*, 2001). The pathways that lead to the activation of caspases are depending on two main pathways (Zimmermann *et al.*, 2001; Saikumar *et al.*, 1999). The first of these depends upon the participation of mitochondria (receptor-independent pathway) which mainly at caspase-9. The second involves the interaction of a death receptor with its ligand (receptor-dependent pathway) which mainly at caspase-8. Finally, the end result of either pathway is caspase activation, the cleavage of specific cellular substrates, the morphological and biochemical changes and death.

1.2.3.1 The receptor-dependent pathway

The receptor-dependent pathway is controlled by extracellular death signals and death receptors. The TNF receptor family, such as Fas receptor (FasR) and Tumor necrosis factor receptor (TNFR) is a best example apoptotic signaling pathways. The cytoplasmic region of these receptors contain cytoplasmic domains of approximately 80 amino acids that are essential for generating death signals, and, as such, they are designated as "death domains" (Boldin *et al.*, 1995; Hofmann and Tschopp, 1995). After ligand-receptor interaction, the death receptors are form homotrimeric complexes and involve homophilic interactions between death domain of death receptors and death domains of the adapter proteins. In the case of TNFR1, which contains death receptor 3 (DR3), interact with TNFR-associated death domain protein [TRADD], whereas FasR, which contains DR4, interact with Fas-associated death domain protein [FADD] (Hsu *et al.*, 1995; Chinnaiyan *et al.*, 1995). Then, the interaction of these lead to recruit the FADD like IL-1β-converting enzyme (FLLICE) or/and Fas-associated huge protein (FLASH), which contain "death effector domains" (DEDs) to from death-inducing signaling complexes (DISC) (Hennino *et al.*, 2000; Imai *et al.*, 1999; Medema *et al.*, 1997). The forming of DISC recruits procaspase-8, which binds via its DEDs, to interaction and results in procaspase-8 activation, which, in turn, further activates downstream caspase-3, -6, and –7, and leaded to cell death by apoptosis (Imai *et al.*, 1999; Nicholson and Thornberry, 1997; Ashkenazi and Dixit, 1998). In addition, Caspase-8 may also cleave Bid, which migrates to mitochondria and releases cytochrome C, thereby setting in motion events that lead to apoptosis via caspase-9 (Saikumar *et al.*, 1999).

1.2.3.2 The receptor-independent pathway

The receptor-independent pathway is mainly control via p53-mediated mechanism and mitochondrial pathway. The dead signals of this are extracellular death signals such as hypoxia, drugs and radiation, and are intracellular death signals such as DNA damage and unrestrained E2F activity (Saikumar *et al.*, 1999; Lundberg and Weinberg, 1999). When DNA damage, at G2 checkpoint, the members of the PI-3K family become activated and directly phosphorylate p53 by Chk1 and Chk2 kinases, through ATM/ATR and DNA-PK dependent signaling (Pietenpol and Stewart, 2002; Canman and Lim, 1998; Hirao *et al.*, 2000; Matsuoka *et al.*, 1998). The result of activated p53 by phosphorylation or directly induced e.g. drugs is activating p53-dependent signaling pathway and leads to two responsive ways, growth arrest and apoptosis. The growth arrest that come from p53-dependent signaling contributes to maintenance of the G2 cell cycle arrest by up-regulating the 14-3-3σ protein and GADD45 that binds to Cdc2 and sequesters the kinase in the cytoplasm (Lopez-Girona *et al.*, 1999; Wang et al., 1999). Moreover, the p53-dependent transcription also elevates the Cdk inhibitor p21, which binds to cyclin/Cdk complexes to reduce phosphorylation of pRB that block biosynthesis of cyclin B1 and Cdc2 (Lundberg and Weinberg, 1999). The induced apoptosis that resulted by p53-dependent signaling inhibit Bcl-2 function through transactivation of Cdc42 (Thomas *et al.*, 2000) and up-regulate Bax expression (Miyashita and Reed, 1995). The Bcl-2 is an anti-apoptotic protein which functions through forming heterodimers with pro-apoptotic proteins such as Bax, thereby inhibiting the mitochondria membrane permeabilization and release of cytoochrome C (Otter *et al.*, 1998; Yang *et al.*, 1997). The Bax (and other pro-apoptotic Bcl-2 family e.g. Bid and Bad) are pro-apoptotic proteins. They function through inducing the mitochondria membrane permeabilization and the release of cytochrome C (Kluck *et al.*, 1997). When cytochrome C from mitochondria released, it binds to the apoptosis protease-activating factor 1 (Apaf-1) and activate them. Then, Apaf-1 binds to procaspase-9 and activates this protease, which in turn activates downstream caspase-3, 6, and 7, and leaded to cell death by apoptosis (Zou *et al.*, 1997; Nicholson and Thornberry, 1997; Ashkenazi and Dixit, 1998).

Schematic representation of apoptotic stimuli, signaling pathways, and effector mechanisms of the receptor-independent pathway and the receptor-dependent pathway are shown in Figure 1.3.

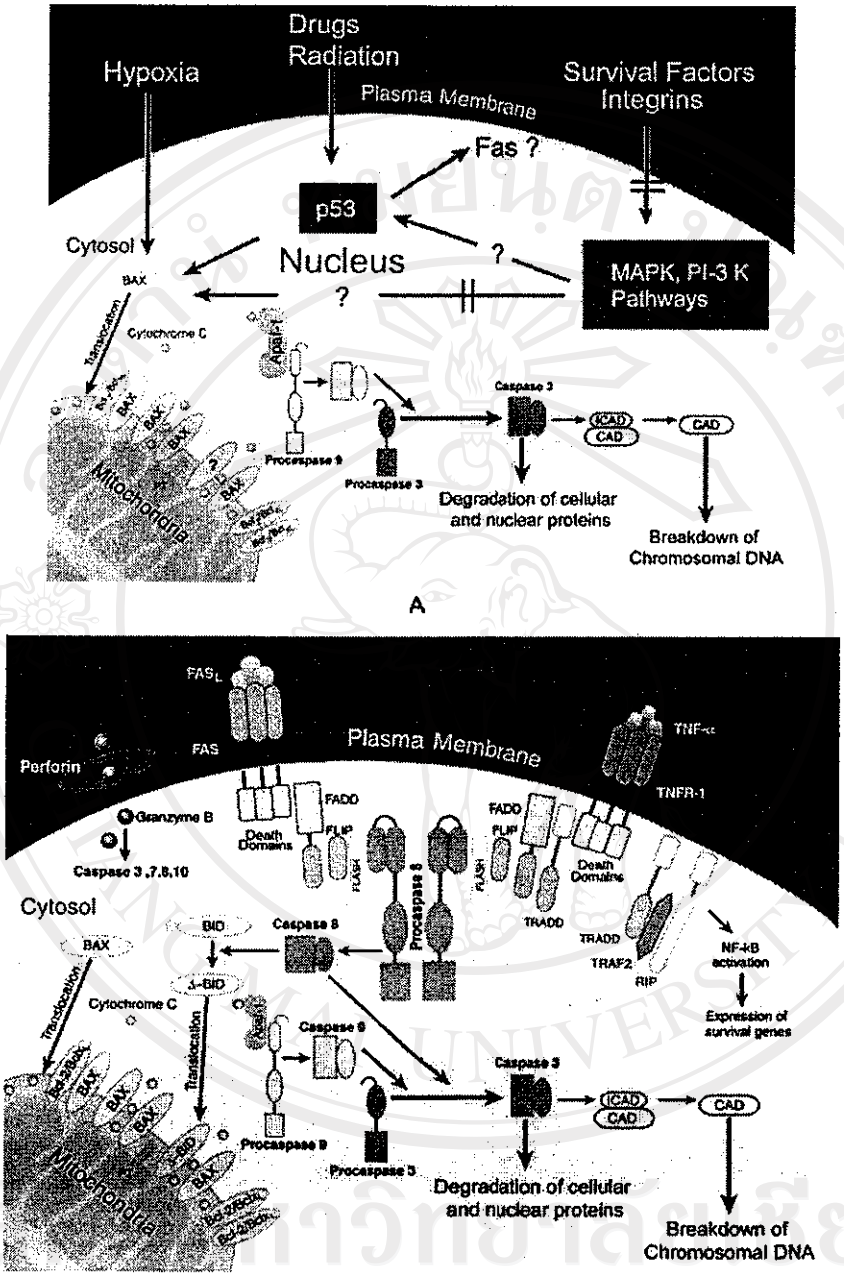

Figure 1.3 Schematic representation of apoptotic stimuli, signaling pathways, and effector mechanisms. (a) The receptor-independent pathway. (b) The receptor-dependent pathway. (Saikumar *et al.*, 1999)

1.2.4 Leukocyte surface molecules

All cells, including prokaryotic and eukaryotic cells, are surrounded with membrane. Cell membrane contains several types of proteins called membrane proteins. The membrane protein is a protein molecule or assembly of molecules that is either embedded in or weakly attached to the biological membrane with which it is associated. Membrane proteins can be divided into 2 types, peripheral membrane proteins and integral membrane proteins. Peripheral membrane proteins are proteins that adhere only loosely to the biological membrane. These molecules do not span the lipid bilayer core of the membrane, but attach indirectly, typically by binding to integral membrane proteins. Integral membrane protein is a protein molecule or assembly of protein that embedded in the membrane (Wikipedia, 2003).

As same as other cells, leukocytes express distinct assortments of molecules on their cell surfaces, many of which reflect either different stages of their lineage-specific differentiation or different states of activation or inactivation. Because, the leukocyte surface molecules are routinely detected with anti-leukocyte mAbs. So, if using different combinations of mAbs, it that possible to chart the cell surface immunophenotypes of different leukocyte subpopulations. The abbreviations have been used for identifying different leukocyte cell surface molecules via historically three conventions (Seare, 2002). The first convention, cell surface molecules are named according to a particular function affected by an anti-leukocyte mAb. For example, the lymphocyte function-associated antigen 1, or LFA-1, was named because antibodies recognizing this structure interfere with lymphocyte cell adhesion events and optimal lymphocyte function. For the second convention is effectively no convention at all. Molecules are named arbitrarily according to individual laboratory preferences. For example, no obvious logic follows in the designations B7 and B220, except that the leading "B" reminds us that these antigens are typically expressed on B lymphocytes. The third conventions, because of these first and second conventions are uniform nomenclature system. In order to resolve the confusion of uniform nomenclature system that initially for human leukocytes, the cluster of differentiation (CD) antigen number has been used to identical and unique reactivity pattern with different leukocyte populations (Seare, 2002). They process that begins with a researcher identifying a molecule on the cell surface of whatever type of immune cell (not just lymphocytes) that they are investigating. Once identified the new molecule is given a tentative label which begins "CDw".

The "w" stands for "workshop" and indicates the label has not yet been confirmed. It may be that the researcher has found a cell surface molecule that someone else already found so a few years must lapse before the CDw label is changed to a true CD designation that confirmed by an international committee (McElwee, 2002).

1.2.5 CD147 molecule

CD147 molecule is a leukocyte surface protein which was designated at the 6th International Workshop on Human Leukocyte Differentiation Antigen (HLDA workshop) (Stockinger *et al.*, 1997). The molecule also known as basigin (Miyauchi *et al.*, 1991), M6 (Kasinrerk *et al.*, 1992) and extracellular matrix metalloproteinase inducer (EMMPRIN) (Biswas *et al.*, 1995). The CD147 molecule is broadly expressed on hemopoietic and non-hemopoietic cell lines, endothelial cells and human peripheral blood cells (Stockinger *et al.*, 1997; Kasinrerk *et al.*, 1999). The molecule is more strongly expressed on thymocytes than on mature peripheral blood T-cells (Kirsch *et al.*, 1997). Peripheral lymphocytes is not significantly express CD147 however its expression is up regulated on activated T-cells which is observed on the surface of lymphoblasts after 3 days of PHA stimulation (Kasinrerk *et al.*, 1992; 1999). A high induction was also observed in neoplasms of the bladder, liver and lung (Muraoka *et al.*, 1993; Staffler and Stockinger, 2000). By using COS cell expression system, Kasinrerk *et al.* (1992) cloned a cDNA encoding M6 protein. Comparison of the M6 sequences with other molecules indicated that M6 is the species homologue of rat OX-47 antigen (Fossum *et al.*, 1991), a mouse molecule termed gp42 (Altruda *et al.*, 1989) or mouse basigin (Miyauchi *et al.*, 1990) or CE9 (Nehme *et al.*, 1995) antigen, and a chicken antigen HT7 (Seulberger *et al.*, 1990), 5A11 antigen (Fadool *et al.*, 1993) or neurothelin (Schlosshauer *et al.*, 1990).

Cloning and sequencing analysis of the CD147 molecule showed that the human CD147 molecule codes for 269 amino acid residues and have a typical feature of a type I integral membrane protein. It is a member of immunoglobulin superfamily with an approximate molecular weight of 50-60 kDa (Kasinrerk *et al.*, 1992; Stockinger *et al.*, 1997). The extracellular domain consists of two Ig-like domains most probably of the C_2 type when determined by comparison with other Ig domains. Domain 1 of the molecule is homologous to domain 3 of IL-1 receptor and domain 2 was found to be significantly related to domain 5 of a

chain of CD22 (Kasinrerk *et al.,* 1992). Endoglycosidase F treatment of immunoprecipitates resulted in a mobility shift from 54 kDa to 28 kDa showed that the majority of the oligosaccharide chains are N-linked (Kasinrerk *et al.,* 1992). The 21 amino acid of the putative transmembrane region are absolutely identical in the human, rat and chicken homologues and, with the exception of one amino acid, also in the mouse and rabbit forms (Kasinrerk *et al.,* 1992; Schuster *et al.,* 1996). Interestingly, the hydrophobic stretch of the transmembrane region of M6 is interrupted by a charged residue, a glutamic acid, and contains a leucine-zipper that are potential protein-protein interaction motifs. This and the strong conservation of the molecule suggest an important functional role for this region perhaps in interactions with other proteins within the plasma membrane or signal transduction. In additon, the CD147 bears high-frequency Oka blood group antigen (Spring *et al.,* 1997). The CD147 gene has been mapped to band p13.3 of chromosome 19 (Kaname *et al.,* 1993) and the mouse gene has been found to consist of seven exons (Cheng *et al.,* 1994).

The molecular function of CD147 molecule in regulation of T cell function is not clearly understood. Previous studies showed that CD147 seems to directly bind to interstitial collagenase (Guo *et al.,* 2000) suggesting the involvement of CD147 in regulating stromal matrix metalloproteases. Therefore, CD147 was termed EMMPRIN (Extracellular Matrix Metalloproteinase Inducer) by Chitra Biswas and colleagues (Biswas *et al.,* 1995). The CD147 monoclonal antibodies (mAbs) AAA6 and UM-8D6 inhibited homotypic aggregation of the estrogen-dependent breast cancer cell line MCF-7, as well as MCF-7 cell adhesion to type IV collagen, fibronectin and laminin (Staffler and Stockinger, 2000), The mAbs to the chicken homologue reduced retina cell aggregation (Fadool *et al.,* 1993). Furthermore, anti-CD147 mAbs could activate the homotypic cell aggregation of U937 cell line by LFA-l/ICAM-1 dependent pathway (Kasinrerk *et al.,* 1999) through the activation of protein kinases and reorganization of the cytoskeleton (Khunkeawla *et al.,* 2001).

CD147 had been found to be co-immunoprecipitated with a_3p_1 and a_6p_1 integrins (Berditchevski *et al.,* 1997). It is therefore tempting to speculate that this physical association underlies a functional cooperation. Co-localization of CD147 with the monocarboxylate transporters MCT1 and MCT4 has also been reported (Juel *et al.,*1999). Studies employing a CD2-CD147 chimera implicate the transmembrane and cytoplasmic domains of CD147 in this interaction (Kirk *et al.,* 2000).

Involvement of CD147 molecule on the regulation of T cell functions has been reported. Earlier studies showed that the mAbs to CD147 molecule had no effect on T cells proliferation (Kirsch *et al.*, 1997). In contrast, one anti-CD147 mAb, MEM-M6/6, was found to prevent human T cell proliferation in allogeneic mixed lymphocyte responses as well as in T cell receptor (TCR)/CD3 plus CD28 driven highly purified T cell cultures (Koch *et al.*, 1999). The discrepancy of the effect of anti-CD147 mAbs in T cell activation may arise from the reactivity of mAbs to different epitopes on the CD147 molecule.

1.3 Objectives

1. To study the epitopes that are recognized by the generated anti-CD147 monoclonal antibodies
2. To search for the molecule association to the CD147 molecule
3. To study the role of CD147 molecule involving on regulation of lymphocyte
4. To study the role of CD147 molecule on the induction of apoptosis