INTRODUCTION

Traditional forms of cancer treatments include the use of surgical resection, chemotherapy and radiation therapy, with the latter two focusing directly on the ability to inhibit DNA replication and cell division ***(Atkins and Gershell, 2002)***.

Generally, these conventional forms of treatment do not specifically target the tumor itself directly. However, with the increasing knowledge in the fields of immunology and molecular biology and its applications, the area of tumor immunology, more specifically cancer immunotherapy, has emerged and newer forms of cancer treatments are being studied to address the issue of tumor specificity ***(Tuting et al., 1997; Davis et al., 2003)***.

The use of cancer vaccines allows one to target specific defined antigens expressed on tumor cells and to induce antitumor immune response. These developments in tumor immunology have stemmed from our increased understanding of how the immune response works and the identification of tumor antigen.

The immune system is comprised of various players on a team, divided into both the innate and the adaptive arms of immunity. The innate immune response is non-specific and is involved in surveying microenvironment to provide signals to work in collaboration with the adaptive immune response. The

generation of effective antigen-specific adaptive immune responses requires presentation of the antigen, the selection and activation of T cells that differentiate to provide various effector mechanisms to eliminate tumor cells.

It is also important to note that tumors have also evolved various active and passive immune escape mechanisms to evade the immune system ***(Ahmad et al., 2004; Campoli et al., 2005)***.

AIM OF THE WORK

To determine the objective response, survival rate, efficacy and feasibility of immunologic effects of autologous immune enhancement against advanced solid tumors.

INTRINSIC IMMUNE PROCESS AGAINST CANCER

1- Antigen presenting cells

There are three types of antigen presenting cells (APC) involved with the uptake of antigen and presentation of epitope peptides to naïve T cells for activation. Each of them is involved in generating different types of immune responses. Dendritic cells (Dendritic cell) are one of the most powerful professional APC in the body. Dendritic cell are characterized by their efficient uptake of antigen, constitutive expression of major histocompatibility complex (MHC) class II molecules and costimulatory molecules, such as B7-1 (CD80), and play a vital role in immune responses ***(Banchereau and Steinman 1998)***.

B cells also constitutively express MHC class II molecules but only express costimulatory molecules following activation. On the other hand, macrophages only express MHC class II and costimulatory molecules upon activation. These three types of APC differentially express MHC class II molecules and costimulatory molecules and they are involved in different aspects of generating an immune response ***(Abbas et al., 2000)***.

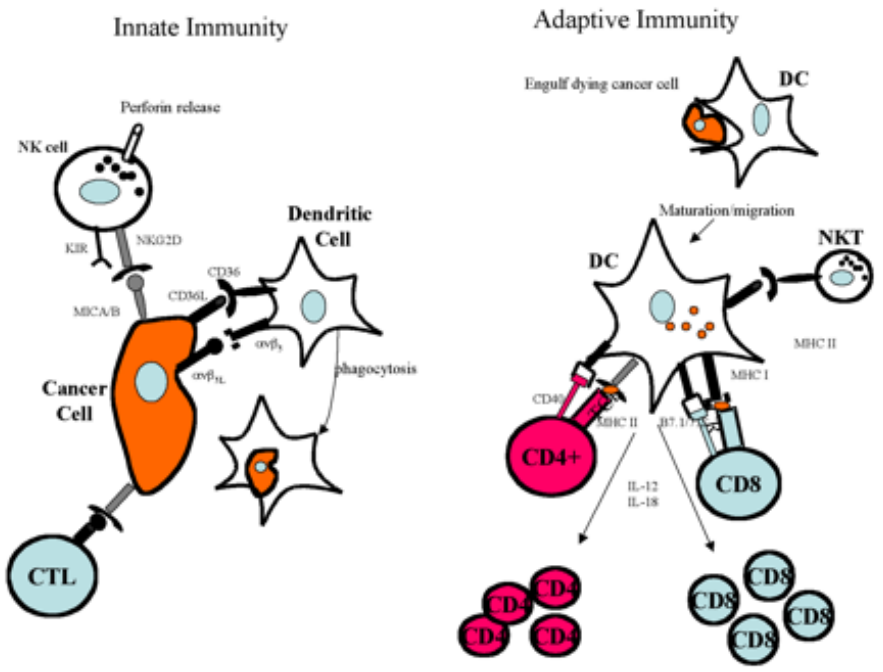

Figure (1): APC activation once these cells are activated there is further cytoxicity and secretion of cytokines to potentiate the immune response toward the tumor cells ***(Schumacher et al., 2006)***

Two main pathways for antigen presentation have been categorized for the loading of antigen peptides on MHC molecules to activate specific subsets of T cells. These pathways are (i) the endogenous pathway and (ii) the exogenous pathway for peptide presentation on MHC class I and II molecules, respectively.

2- Endogenous pathway

In the endogenous pathway, intracellular antigen and proteins are transported from the cytoplasm into the endoplasmic reticulum by first passing through the proteasome for proteolytic cleavage. Proteins are targeted to the proteasome

by ubiquitination then unfolded and cleaved into peptides 8-12 amino acids in length.

These peptides are transported into the endoplasmic reticulum (ER) with the aid of a membrane protein called transporter associated with antigen processing (TAP). The peptides in the ER are loaded into the binding grooves of MHC class I molecules, consisting of the class I α chains and β_2 microglobulin, and the interaction stabilized.

MHC class I-peptide complexes are transported from the endoplasmic reticulum to the cell surface where they interact with the T cell receptor of $CD8^+$ T cells. The expression of MHC class I molecules is not restricted, as all nucleated cells express it on their cell surfaces ***(Germain 1995; Abbas et al., 2000)***.

3-Exogenous pathway

The exogenous pathway is involved with the uptake of captured antigen from the extracellular environment and internalization through endocytosis or phagocytosis. The proteins are enzymatically degraded in the formed endosomes and lysosomes to generate peptides of 10 to 30 amino acids in length.

These generated peptides then interact with the newly synthesized MHC class II molecules, consisting of α and β chains, to form a stable MHC class II-peptide complex. The

stabilized complex is then transported to the cell surface for expression and potential interaction with the T cell receptor on $CD4^+$ T cells ***(Germain 1995; Watts, 2004)***.

Expression of MHC class II molecules is limited exclusively to cells with antigen presentation capabilities such as dendritic cells. It is also important to note that the fate of antigen presentation of exogenous Antigen is not limited to MHC class II molecules but also has the ability to be cross-presented onto MHC class I molecules ***(Heath and Carbone 2001; Ackerman and Cresswell, 2004)***.

This is done through a process called cross-priming or cross-presentation, first described by Bevan (Bevan 1976), and can stimulate $CD8^+$ T cells to mount an immune response. Some of the mechanisms involved with cross-priming include the regurgitation of peptides and loading onto MHC class I molecules via antigen in the phagolysosomes acquiring access to the cytosol through the use of heat shock proteins (HSPs), direct entry through macropinocytosis and high concentrations of the Antigen ***(Heath and Carbone 2001; Ackerman and Cresswell, 2004)***.

Generating the immune response

The activation of naïve T cells requires the presence of the necessary signals to ensure the appropriate immune response is generated, either a cellular or humoral response. The cellular response is characterized by the generation of

$CD8^+$ cytotoxic T lymphocytes (cytotoxic T lymphocyte) that are able to recognize and lyse tumor cells while the humoral response involves antibodies generated from B cells. The activation of T cells requires a coordinated effort provided by two signals ***(Bretscher and Cohn, 1970)***.

The Antigen-specific interaction between the T cell receptor of naïve T cells with the peptide-MHC molecule complex on APC consists the first signal.

The second signal stems from costimulatory molecules expressed on APC, such as B7-1 (CD80), B7-2 (CD86) and other costimulatory family members, and interacting with its cognate receptor on T cells, such as CD28 for B7-1 and B7-2. For both T or B cells that only interact with the peptide-MHC molecule, but fail to receive a costimulatory signal, the cells are not activated and become tolerant or anergic. Upon receiving the proper signals, the activated cells rapidly proliferate and are able to begin their effector functions. In addition, the activation of T helper cells is central to the development of an immune response by activating antigen-specific effector cells and the recruitment of cells in the innate immune response ***(Hung et al., 1998)***.

1-Cell mediated immunity

Cell mediated immunity or cellular response, involves the generation of cytotoxic T lymphocytes that effectively and specifically lyse their target cells. Cell mediated immunity is

generally regarded as the most favorable effective antitumor immune response.

The cytotoxic T lymphocyte response can be generated through a T helper cell-dependent and independent process. The T helper cell-dependent process requires the presence of $CD4^+$ T-helper 1 cells, upon receiving the appropriate signals, for the interactions between the APC and both $CD4^+$ T-helper 1 and $CD8^+$ T cells. This is important because tumor cells generally do not express MHC class II; however, this is not absolute as a significant proportion of melanoma cells in tumors have been shown to express one or more MHC class II genes ***(Altomonte et al., 2003)***.

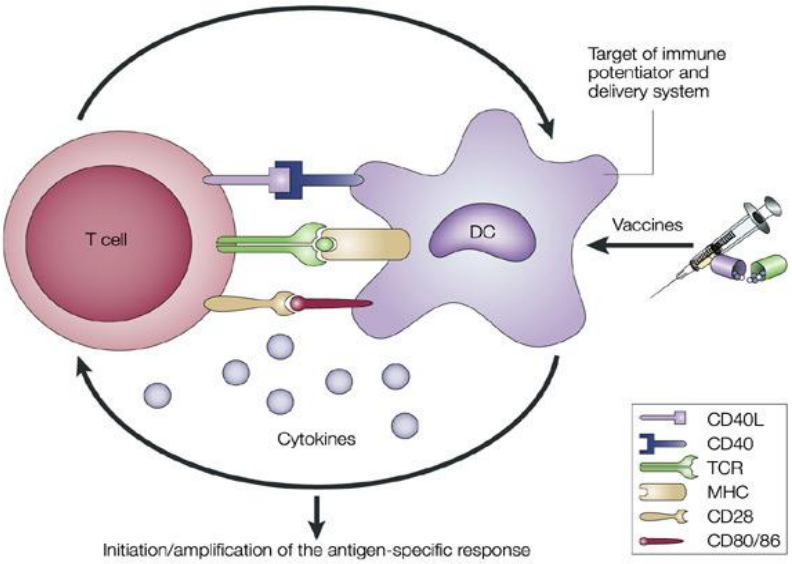

Figure (2): Antigen-presenting cells serve as the bridge between innate and antigen-specific responses

The interactions between antigen-presenting cells (APCs) and antigen-specific T cells initiate and amplify pathogen-specific responses. The key interaction is driven by the recognition of antigenic peptide–major histocompatibility complex (MHC) dimers by T cells bearing T-cell receptors (TCRs) with high affinity for the complex. However, this signal alone is not sufficient for initiation and amplification of specific T-cell responses. Co-stimulatory signals and the production of pro-inflammatory cytokines, provide the 'infectious context' by which the full activation of antigen-specific T cells is achieved. The expression of co-stimulatory molecules and cytokines by APCs is tightly regulated and induced only when the APC encounters antigens associated with pathogen-associated molecular patterns ***(Derek et al., 2003)*.**

Activated T-helper 1 cells also express CD40 ligand (CD40L) that interacts with CD40 expressed on APCs, including Dendritic cells, to further enhance their effector functions by up-regulating the expression of pro-inflammatory cytokines and important cell surface molecules such as MHC and B7-1 molecules.

T-helper 1 cells are also able to produce interleukin-2 (IL-2) and interferon-γ (IFN-γ) to provide support for clonal expansion and differentiation of $CD8^+$ T cells, either directly or indirectly ***(Knutson and Disis, 2005)***.

Generation of $CD8^+$ T cell response via T-helper 1 cell-independent methods can also be induced, as seen in virus-infected cells, whereby the APC directly stimulates the $CD8^+$ T cell and by passes the need for coordinated help from T-helper 1 $CD4^+$ cells.

Once the activated T cells have proliferated and differentiated into effector T cells, $CD8^+$ cytotoxic T lymphocyte cells are able to recognize cells displaying a specific epitope on MHC class I molecules and mediate cytolysis through the release of cytotoxic granule proteins, mainly perforin and granzymes.

Another mechanism of cytolysis involves the interaction of Fas ligand (FasL), expressed on cytotoxic T lymphocyte, interacting with the target protein Fas to result in apoptosis of the target cell. Previously, the effector functions for T-helper 1 cell were once thought to only provide the necessary help required in activating cytotoxic T lymphocytes; however, T-helper 1 cells are also able to recognize target cells. This results in direct cytolysis through FasL-Fas pathways, TNF-related apoptosis-inducing ligand (TRAIL) and the release of cytokines inducing cytotoxicity ***(Knutson and Disis, 2005)***.

2-Humoral immunity

B cell activation resulting in the production of Antibodies is characteristic of humoral immunity that specifically recognizes the Antigen. B cells have the ability to process

Antigen and present short-length peptides bound to MHC class II molecules.

The $CD4^+$ T-helper 2 cells recognize the peptide-MHC class II complex on B cells and the cells become activated with costimulation. Activated T-helper 2 cells express CD40L that is able to interact with constitutively expressed CD40 on B cells in germinal centers. T-helper 2 cells also secrete characteristic cytokines such as IL-4 and IL-10, and determine the immunoglobulin (Ig) subclass produced.

The IgG1 subtype is normally associated with the humoral response, whereas the presence of IFN-γ induces class switching of the antibodies to IgG2a. The IgG2a subtype is commonly associated with cell mediated immunity response. B cells proliferate and undergo somatic mutation of the Ig genes, to end up with B cells producing Antibodies with the highest affinity for the antigen.

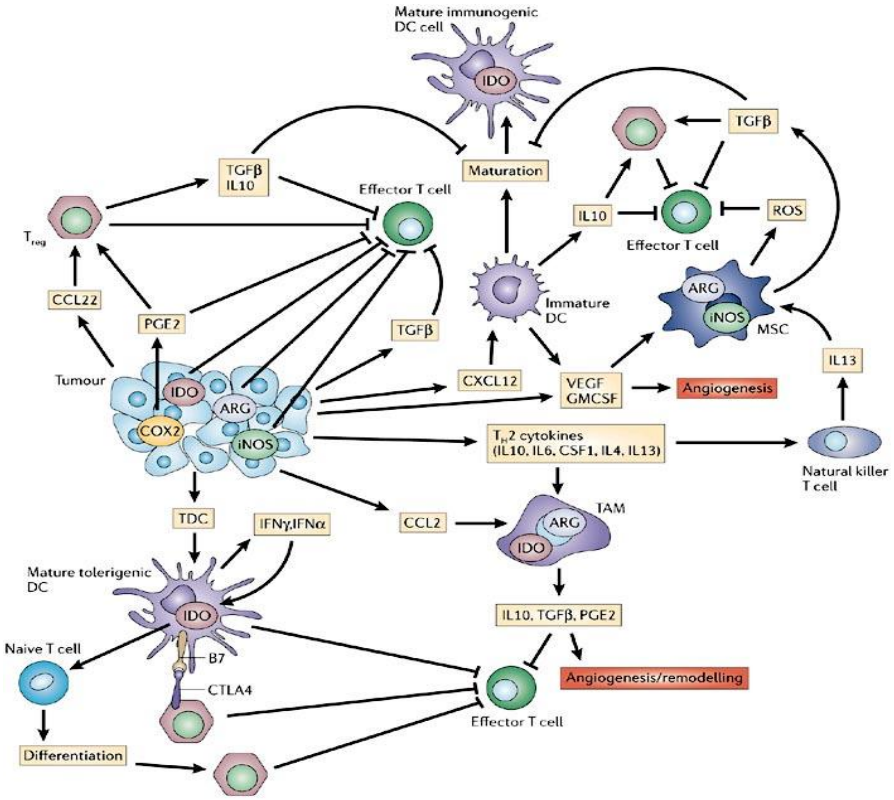

Figure (3): Interactions between the tumour and cells of the immune system that foster an immunosuppressive microenvironment

Through the production of cytokines (such as interleukin 10 (IL10), transforming growth factor β(TGFβ), IL13 and vascular endothelial growth factor (VEGF)) and chemokines (such as CCL22, CCL2 and CXCL12), tumours can promote the migration and expansion of cells that negatively regulate the immune system. T-regulatory (T_{reg}) cells, which block T-effector cells through both direct interaction and the production of immunosuppressive cytokines such as TGFβ and IL10, can be recruited as well as myeloid suppressor cells (MSCs), immature dendritic cells, natural killer T cells (NKTs) and

tumour-associated macrophages (TAMs). These cells act to suppress the proliferation of effector-T cells (such as $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells), the production of cytokines (such as (IFN) and IL2), and cytolytic activity. Both tumour cells and host cells can express enzymes that are involved in immune suppression (such as arginase (ARG), indoleamine 2,3-dioxygenase (IDO), cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS)) ***(Alexander, 2006)***.

Antibodies generated would be antigen specific and mediate their effects through a variety of effector mechanisms such as complement dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. Antibody-dependent cell-mediated cytotoxicity involves macrophages and natural killer cells mediating the cell cytotoxicity effects through the recognition of a receptor on the constant Fc portion of the Ig.

3-Tumor Antigen

The ideal tumor Antigen would be specifically expressed only on tumor cells while completely absent in normal cells and deemed critical for cancer cell survival. This is not the case, as early classification schemes grouped tumor antigens into two categories: (i) tumor specific Antigens and (ii) tumor associated ntigens (TAA).

The definition of tumor specific antigens was used to identify antigens that were uniquely and solely expressed in tumor cells. On the other hand, TAA was defined as being

expressed in normal cells but aberrant or dysregulated expression observed in tumors. This suggested that TAAs were of lower immunogenicity and patients with tumors were already tolerized to the antigen. For the tumor antigens identified, very few TSA have been identified with a fair majority of the tumor Antigen being TAA identified.

With newer methods available for molecularly identifying and characterizing tumor antigens, modifications to the classification scheme have been made over time; taking into consideration that majority of the tumor Antigens were TAAs.

The new classification scheme was devised based upon the function or the expression pattern of the antigen and classified into five different categories as follows: ***(Abbas et al., 2000; Renkvist et al., 2001; Davis et al., 2003; Novellino et al., 2005).***

(i) Differentiation Antigen, which includes the well-studied melanocyte antigens tyrosinase, glycoprotein-100 (gp100), Melan-A/melanoma antigen recognized by T cell (MART-1) Antigen, prostate specific Antigen (PSA), prostate-specific membrane Antigen.

(ii) Cancer-testis Antigen or oncofetal antigen, where the TAA expression is limited in normal tissues to gametes and trophoblasts but becomes aberrantly expressed in a wide variety of cancers. Some examples include melanoma antigen-1 (MAGE-1), MAGE-3, carcinoembryonic antigen and alpha-fetoprotein .