



Reviewing the Concepts of Immune Surveillance of Cancer Cells, Immunogenic Cancer Cell Death and the Role of Medicinal Plants in Anticancer Immunity

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ABSTRACT

Current standard treatment of cancer usually involves surgical resection, chemotherapy and radiotherapy. Surgical resection is aimed at eliminating the primary tumour from its anatomical site of origin, while chemotherapy and radiotherapy are meant to target metastatic tumour cells around the primary tumour site and distant parts of the body. Although standard treatment of cancer usually produces some initial positive clinical responses, such responses are often followed by recurrence of malignancy, several months or years later. Consequently, efforts are currently being focused on stimulating a patient's natural immune responses, to fight against metastasized cancer cells that are mainly responsible for cancer relapse. The role of the immune system in the prevention and treatment of cancer has long been established. In fact, a patient's response to chemotherapy and radiotherapy has been observed to be largely dependent on the level of competence of the immune system. Despite these observations, little effort is currently being made to boost a patient's immune response during cancer treatment. The confidence and consequent use of medicinal plants for treatment of many disease conditions is currently growing rapidly and many of these medicinal plants have been observed to possess anti-neoplastic, anti-malignant, immune-stimulatory, and cytoprotective effects, which can all be harnessed, to produce better

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outcomes for cancer treatment. This review therefore seeks to reawaken the interest of researchers, pharmaceutical giants, clinicians and scientists in investigating and validating the potential use of extracts of medicinal plants as valuable components in the management and treatment of cancer.

Keywords: Immune surveillance; immunogenicity; cancer cell death; medicinal plants; anticancer immunity.

1. INTRODUCTION

The immune system functions principally to protect its host against environmental agents such as toxic chemicals and microorganisms, in order to maintain the body's integrity [1]. Individuals with compromised immune responses are therefore highly susceptible to serious and sometimes life-threatening infections caused by these agents [2].

The role of chronic infections, especially viral infections, is well established in the pathogenesis of various human neoplasms, including prostatic carcinoma, colorectal cancers [3], cervical cancers, B-cell lymphoma, hepatic carcinomas and Kaposi sarcoma [4]. Chemical carcinogens also play important roles in the aetio-pathogenesis of cancers and there is massive evidence to suggest that the immune system does respond to cancer cells.

The immune system functions by recognizing and responding to antigens present on the surfaces of cells and microorganisms as well as toxic chemical substances which can also serve as antigens. Toxic chemical substances and cells bearing such antigens are often recognized and destroyed by a competent immune system, thus protecting the body from harm [5]. Different classes of lymphocytes often recognize specific antigens expressed on cell surfaces and differentiate to form effector cells which are involved in the elimination of such surface antigens. While B lymphocytes differentiate into antibody-secreting cells that neutralize microbes and activate the complement system, T lymphocytes on their part, respond by either secreting cytokines (helper T lymphocytes) or directly killing antigen-infected cells (Cytotoxic T lymphocytes).

Conventional treatment of cancer by surgery, chemotherapy and radiotherapy has been observed to be more effective in patients with competent immune systems, when compared to those with compromised immune responses [6]. This suggests the need to optimize a patient's

immune responses, while undergoing radiotherapy and chemotherapy, which however, is not usually the case. Over the years, studies have revealed that many medicinal plants possess both anticancer and immune-stimulatory activities. Consequently, many conventional anticancer agents currently in use were isolated from medicinal plants, whose immune-stimulatory effects have been largely ignored or under reported. Apart from reviewing the role of the immune system in the fight against cancer, this paper also seeks to highlight the immunomodulatory activities of many medicinal plants, whose extracts have also been observed to possess potent anticancer activities. It further emphasizes the need to harness these anticancer and immune-stimulatory activities of popular medicinal plants and bring them to the centre stage, in the management and treatment of cancers.

2. IMMUNE SURVEILLANCE OF CANCER CELLS – HISTORICAL PERSPECTIVE

The discoveries that led to the establishment of the concept of cancer immunotherapy can be divided into several eras such as the early, intermediate and modern eras, based on sequence of discoveries.

2.1 The Early Era

The concept of immune surveillance of cancer cells was originally envisioned by Erlich in 1909 but later coined by Burnet (1949) and James (1950s). Erlich proposed the "magic bullet" strategy, whereby soluble factors which we now refer to as antibodies, deliver toxins directly to malignant cells, leading to their recognition, killing and consequent control of cancer growth [7]. Immune surveillance suggests that both precancerous and malignant cells are capable of stimulating immune responses that result in the destruction of malignant cells. Burnet and Thomas believed that the immune system protected against nascent cancers by eliminating malignant cells even before they become detectable as tumours [8].

Three basic mechanisms used by the immune system to prevent the development of cancers have been identified. In the first instance, the immune system protects its host from virus-induced cancers such as colorectal cancer, cervical cancer, B-cell lymphoma, liver cancers and Kaposi sarcoma by suppressing or eliminating such viral infections. Secondly, the timely intervention of the immune system ensures that pathogens are promptly eliminated and inflammation resolved in order to prevent the establishment of a chronic inflammatory environment which is conducive for tumour growth. The immune system can also recognize and kill tumour cells expressing some tumour-specific antigens or molecules that are induced by cellular stress such as starvation, cytotoxic agents, reactive oxygen species and DNA damage. This third mechanism constitutes the concept called 'immune surveillance' [9].

The original immune surveillance theory as envisioned by Erlich and proposed by Burnet and Thomas in the early and mid-nineteenth century later got a boost, following the discovery of lymphocytes as mediators of immune response. In 1959, Lederberg J. proposed the self-non-self-discrimination theory, in which it was suggested that lymphocytes are the mediators of tumour surveillance which protects immune competent hosts against tumours [10]. Lederberg's theory suggested that since most cancers occur after maturation of the immune system, any tumour-specific antigen expressed on cancer cells is easily recognized as non-self. The immune system therefore monitors the body for the development of tumours, which are promptly eliminated as they arise.

Other studies that contributed largely to the immune surveillance theory include those by Bretscher and Cohn, who introduced the Association Recognition or Two Signal model whereby an interaction between antigens and B-lymphocytes generate a first signal (signal one), which is capable of killing or inactivating the affected lymphocyte in the long term, causing it to become refractory to activation unless a second signal is initiated. Complete activation therefore involves a second signal (signal two), triggered by direct or indirect recognition of a second binding site on the antigen by cells which the authors referred to as 'helper lymphocytes'. These helper cells were later observed to be derived from the thymus (T-lymphocytes) and assist B-cells in carrying out their humoral antibody functions [11]. The Two Signal model is now widely accepted and B-cells are now known

to bind and internalize antigens which are then processed and re-expressed in major histocompatibility cell class two (MHC II) molecules. Expressed MHC II molecules are recognized by T-helper cells, which send helper signals using several cytokines such as interleukins -4 and -5 [12].

In 1974, following observations that T-cells responded more effectively against cells from members of the same species than against cells from different species, Lafferty and Cunningham modified the Two Signal model. They suggested the existence of accessory cells, now referred to as 'antigen presenting cells' (APCs). According to Lafferty and Cunningham, helper T-cells, just like B-cells, are not constitutively active but also require two activating signalling, with the second signal being provided by APCs. This second signal was observed to be specie-specific and responsible for the stronger T-cell response against cells from the same species [12].

Furthermore, they proposed that the presence of only signal one results in tolerance of T-cells, indicating that the second signal is critical for complete activation of helper T-cell response, similar to B-cell response. However, a major difference between the helper T-cells and APCs is that while helper T-cells bear clonally distributed antigen-specific receptors and are therefore antigen specific, the same cannot be said of APCs which are not antigen-specific and therefore cannot distinguish between self and non-self.

These studies and observations that stemmed largely from the original immune surveillance theory all failed to detail the mechanisms through which cancer cells escaped detection in immune competent individuals. This single shortcoming continued to cast doubt in the minds of some researchers until 1974, when the validity of the concept of immune surveillance was challenged by Osias Stutman and colleagues, giving birth to the intermediate era.

2.2 The Intermediate Era

The intermediate era was characterized by studies that suggested the non-existence of cancer immune surveillance, especially for spontaneous and chemically-induced cancers. Stutman and colleagues carried out experiments by injecting athymic nude mice and normal mice with the chemical carcinogen called 3-methylcholanthrene (MCA) at birth and monitoring these mice for tumour development

for 120 days. Results obtained showed no significant differences in the development of local sarcomas or lung adenomas, following administration of MCA. They therefore concluded that although immune surveillance apparently occur in nature and might be effective in protecting against some oncogenic viruses, the concept was however not universal and cannot be applied to all cancer cases, especially chemically-induced cancers.

Other studies similar to those of Stutman et al. quickly followed, including that by Rygaard and Povlsen which used about 10,800 nude mice over a period of three to seven months, to show that there was no significant difference in spontaneous tumour formation between these mice and normal mice. Based on these and other overwhelming evidences, the immune surveillance theory was completely abandoned by the late 1970s. However, a few number of researchers continued in their efforts to validate the concept of immune surveillance.

The discovery of natural killer (NK) cells during the 1970s initially appeared to be a breakthrough for the concept but lack of detailed understanding of their functions posed a major setback [13-14]. However, more recent findings have shown that Stutman's experiments and other similar experiments that used athymic nude mice to dispute the immune surveillance theory cannot be completely relied upon.

Firstly, nude mice are currently known to produce low but observable functional amounts of alpha, B and T-cells and therefore, possess some levels of adaptive immune response. NK cells have also been discovered to be present and function optimally in nude mice, thereby potentiating their innate immunity [13-14]. Also, the very potent effect of the existing innate immunity on adaptive immunity was not considered in these experiments. Furthermore, the enzyme called aryl hydroxylase which metabolises MCA to its carcinogenic form has been discovered to be highly expressed in the CBA/H strain of mice which Stutman studied in his experiments [13-14]. These observations all suggest that the athymic nude mice might be an inappropriate model for an immunodeficiency state [13-14]. Following these discoveries, the re-emergence of the concept of immune surveillance gradually gathered prominence during the 1980s, until the early 1990s when it finally got a second chance to make a massive impact in the field of cancer immunology.

2.3 The Modern Era

Janeway in 1992, proposed that innate immunity, like adaptive immunity, is not constitutively active, suggesting that baseline immunity is non-responsive. He further opined that APCs express pattern-recognition receptors (PRRs) capable of recognizing conserved forms of molecules present only in evolutionary distant species such as bacteria [15]. Binding of such evolutionary distant species to PRRs results in the activation of APCs and consequent ingestion of bacteria and associated antigens. Ingested antigens are then re-expressed as proteins in MHC class II leading to activation of T-cell mediated adaptive immune responses. This concept was therefore appropriately termed the 'infectious non-self and non-infectious self' model.

In 1994, Matzinger P. proposed the 'danger' model, in an attempt to explain how matured and competent immune systems might fail to recognize cancer cells. This model was therefore meant to fill in the gaps present in Lederberg's, Bretscher/Cohn's, Lafferty/Cunningham's and Janeway's models and theories. Among other questions, Matzinger challenged the Self/Non-self-discrimination theory by asking why competent immune systems fail to prevent tumours, even though many cancer cells are known to express proteins that are mutated hence foreign to the body. She also sought answers to why mammalian mothers tolerate their foetuses and lactating breasts, even in the presence of proteins that were not originally considered as self.

The 'danger' model suggests that the competent immune system is more concerned with and therefore reacts to 'dangerous' and potentially destructive signals, whether self or non-self, rather than discriminate between self and non-self. This model indicates that the immune system recognizes and eliminates any cell or molecule that causes tissue damage [16].

Under this model, healthy cells do not send danger signals, whereas, cells that are distressed, damaged and those dying abnormally (by necrosis) usually activate local APCs by sending danger signals also referred to as 'alarm signals' or 'signal zero', resulting in immune responses. In contrast, old and worn-out cells undergoing the process of programmed cell death (apoptosis) send signals referred to as 'eat me' signals that attract and activate neighbouring cells and phagocytes without inducing co-stimulatory immune responses. Some of the

molecules involved in sending Matzinger's danger signals have been identified to include the nuclear protein HBGM-1, uric acid, IFN- α , heat shock proteins, alternatively spliced domains of fibronectin and some endogenous nucleic acids. These molecules are collectively referred to as 'alarmins', some of which utilize the same Toll-like receptors seen in microbial recognition [16].

Although Matzinger's model might suggest that inadequate signalling is responsible for the insensitivity of cancer cells to the immune responses in immune competent individuals, it however did not detail the mechanisms through which such reduced signalling occur that result in cancer cells' evasion of immune surveillance. Nevertheless, results from Matzinger's and other preceding studies have made important contributions to the field of cancer immunotherapy in recent times.

However, more recent discoveries have led to the establishment of the concept of 'tumour escape' in which the mechanisms via which cancer cells escape the surveillance of a competent immune system have been suggested. Alterations in signal transduction molecules such as T-cell receptor zeta chain and p56 have been observed in cancer patients and mice. These alterations are believed to begin at the primary site of tumours and then spread to

distant sites until they become detectable in peripheral blood T-lymphocytes. They were also observed to be non-antigen specific [17].

Several human cancers have also been observed to down-regulate the surface expression of MHC class 1, leading to evasion of cytotoxic T-lymphocyte-mediated recognition and killing [18]. A complete loss of some tumour-specific antigens from cell surfaces also occur in some rapidly proliferating cancer cells, while others lack the expression of co-stimulatory molecules. Some tumour cells are also believed to produce large amounts of immunosuppressive transforming growth factor β (TGF- β) which inhibits the proliferation and activation of lymphocytes and macrophages, thereby allowing cancer cells to thrive. Others express high levels of Fas ligand (FasL) which mediates apoptosis of tumour infiltrating lymphocytes (TIL), thus inhibiting TIL-mediated killing of cancer cells.

These findings cleared a major barrier against the immune surveillance theory and renewed efforts geared towards identifying the basic mechanisms involved in immune surveillance. The next round of experiments in the field of tumour immunology were therefore aimed at providing massive evidence to show that the immune system does respond to spontaneous, infection and chemically-induced cancer cells.

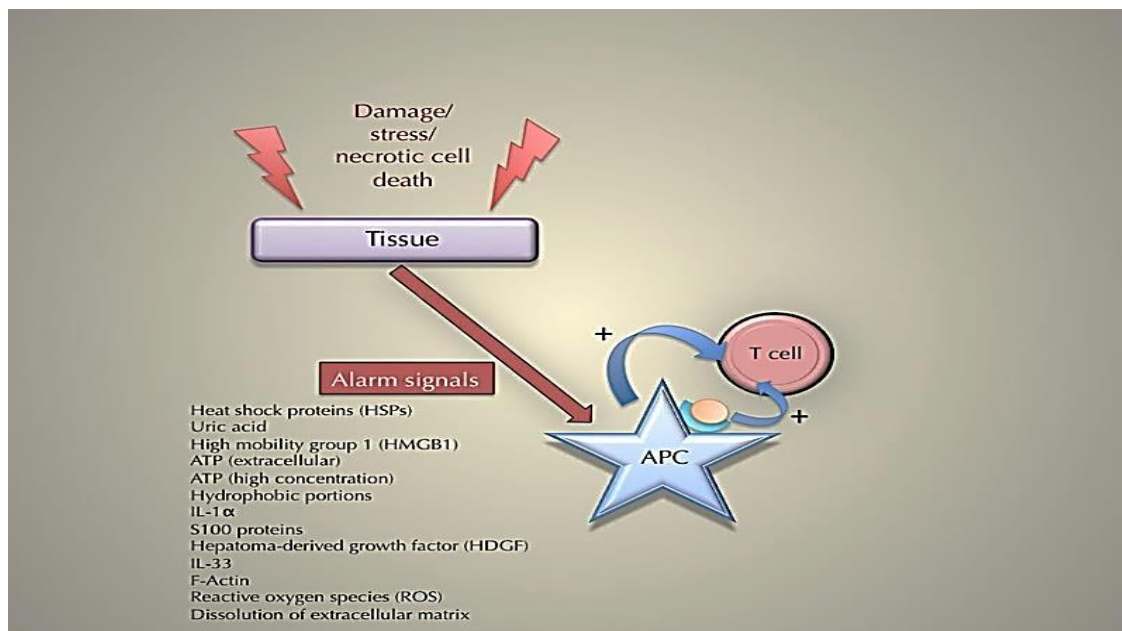


Fig. 1. Stress-induced tissue damage and necrotic cell death lead to release of danger signals (alarmins) which activate local immune responses. (Taken from Pradeu and Cooper, 2012)

3. EVIDENCES OF IMMUNE SURVEILLANCE IN MICE

3.1 Interferon- γ Pathway

Studies done in mice have shown that alterations of the IFN- γ signalling pathway result in defects in the functioning of innate immunity, characterized by marked increase in host susceptibility to a wide range of microorganisms, including viruses [19].

Dighe AS demonstrated in 1994 that endogenously produced IFN- γ is capable of protecting host cells against spontaneous and chemically induced tumours as well as transplanted tumours. Dighe and colleagues transplanted tumour cells into mice and treated these mice with neutralizing monoclonal antibodies against IFN- γ . Transplanted immunogenic sarcomas were later observed to grow faster in these mice, when compared with controls. Further studies also revealed that defects in the IFN- γ receptor or signal transducer and activator of transcription 1 (STAT 1) result in a 10 to 20 fold increase in susceptibility of mice to MCA-induced tumorigenesis. These observations were largely confirmed by independent experiments in which mice lacking the gene that code for IFN- γ were also found to

be more susceptible to tumour formation when compared with controls [20].

3.2 Recombination Activating Genes (RAGs) 1 and 2

Mice that are defective in either of the recombination activating genes (RAGs) 1 and 2 are unable to rearrange lymphocyte antigen receptors and are therefore completely deficient in NK cells, T-lymphocytes and B-lymphocytes. Injection of MCA into such mice has been shown to be associated with an earlier development of MCA-induced tumours with higher frequency, when compared with wild-type mice [21]. RAG 2-deficient mice have also been observed to be highly susceptible to spontaneous development of tumours, especially intestinal malignancies such as intestinal adenomas (35%) and intestinal adenocarcinomas (50%). Combined deficiency of RAG 2 and STAT 1 resulted in an increase in both the incidence and spectrum of tumours to include breast adenocarcinoma (40%) and colon adenocarcinoma (20%), when compared with normal controls [22]. These studies clearly demonstrate that both the innate and adaptive immune systems are actively involved in the prevention of spontaneous and chemically induced cancers.

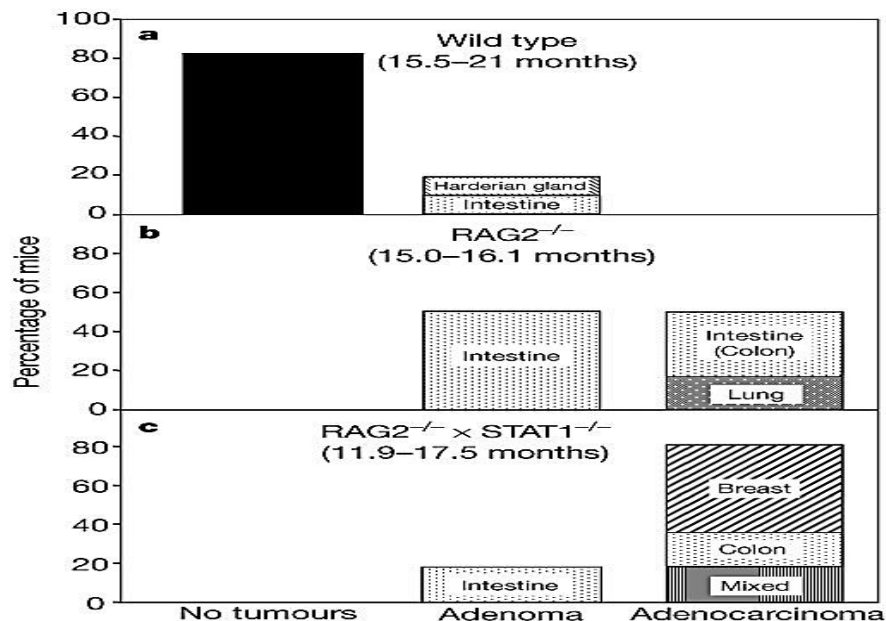


Fig. 2. RAGs 1 and 2-deficient mice show earlier development (15 – 16.1 months) of MCA-induced tumours when compared with wild-type mice (15.5 – 21 months), while combined deficiency of RAG 2 and STAT 1 increases the incidence and spectrum of cancers, to include breast and colon adenocarcinomas (c above). (Shakaran V. 2001)

3.3 Perforin and Trail

Lack of perforin – a cytolytic component of cytotoxic T- and NK- cells that mediates cell-dependent killing of target cells – has also been shown to increase the susceptibility of mice to MCA-induced tumours by 1000 fold, when compared with immune competent mice. Associated defects in p53 were also observed to accentuate the susceptibility of these mice to lymphoma [23].

Tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) is a member of the larger TNF family that initiates apoptosis by interacting with TRAIL-R2 receptors in mice. Smyth et al. demonstrated in 2003 that TRAIL is constitutively expressed in some NK cells of the liver, where it is activated by cells of the immune system such as IFN- α/β , INF- λ and dendritic cells. Activated TRAIL is believed to be involved in NK cell and IFN- λ dependent killing of tumours [24]. Blockage of TRAIL by use of monoclonal neutralizing antibodies has also been observed to cause an increase in the incidence of MCA-induced fibro sarcoma in mouse models, while mice lacking the TRAIL gene show a higher incidence of fibrosarcoma when compared with wild-type mice [25]. These observations suggest that TRAIL is a very potent molecule involved in the immune surveillance of cancer cells.

4. EVIDENCES OF IMMUNE-SURVEILLANCE IN HUMANS

4.1 Immunosuppressive Therapy and Cancers

In humans, there are numerous evidences to suggest that the incidence of malignancy increases several folds, following suppression of the immune system either by drugs (prior to organ transplantation) or by diseases.

Age and sex-matched population-based studies done in the past suggest a 3-fold increase in cancer incidence, following administration of immunosuppressive therapy prior to solid organ transplantation, when compared with the general population [26]. Following organ transplantation, especially kidney transplantation, the incidence of cancer was observed to increase in 23 out of 28 cancer types studied. Although majority of cancers with increased incidences were those of viral origin, others without any infectious cause, including leukaemia, multiple myeloma, melanoma, bladder, kidney, colorectal and

thyroid carcinomas were also found to be significantly increased, suggesting an important and wide-spread function of the immune system in the prevention of cancers, both virus and chemical-induced cancers as well as spontaneous cancers [27].

In non-transplant patients, administration of immunosuppressive therapy for the treatment of autoimmune and collagen vascular disorders have also been widely associated with increased risk of developing cancers. Studies have revealed an 11-fold increase in the incidence of non-Hodgkin lymphoma; 5-fold increase in squamous cell carcinoma; 9-fold increase in primary liver carcinoma and a 4-fold increase in carcinomas of the bladder, following systemic administration of immunosuppressive therapy such as azathioprine, cyclophosphamide or chlorambucin in patients with autoimmune disorders [28].

Furthermore, second malignancies such as carcinomas of the lungs, skin, breast, bladder, pancreas and the colon may also develop in cancer patients, due to the immunosuppressive side effects of cytotoxic anti-cancer drugs [28].

4.2 Immunodeficiency and Cancers

The acquired immune deficiency syndrome (AIDS) is a viral infection caused by the human immunodeficiency virus (HIV) and characterized by a devastating suppression of the immune system of affected individuals. The risk of developing Kaposi Sarcoma (KS), the most common neoplasm in AIDS, has been shown to increase by a hundred fold in AIDS patients, when compared with the general populace. Besides KS, the risk of other cancers such as non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, cloacogenic carcinomas of the anus and embryonic cell carcinomas of the testis are also significantly increased [29]. These findings suggest the effectiveness of a competent immune system in preventing these cancers in immune competent individuals.

Before the advent of AIDS, children with rare congenital immune defects such as ataxia telangiectasia and x-linked gammaglobulinaemia were known to show increased risk of lymphoma, although the extremely low incidence of these conditions did not allow for extensive studies to examine for increased risk of other cancers [30].

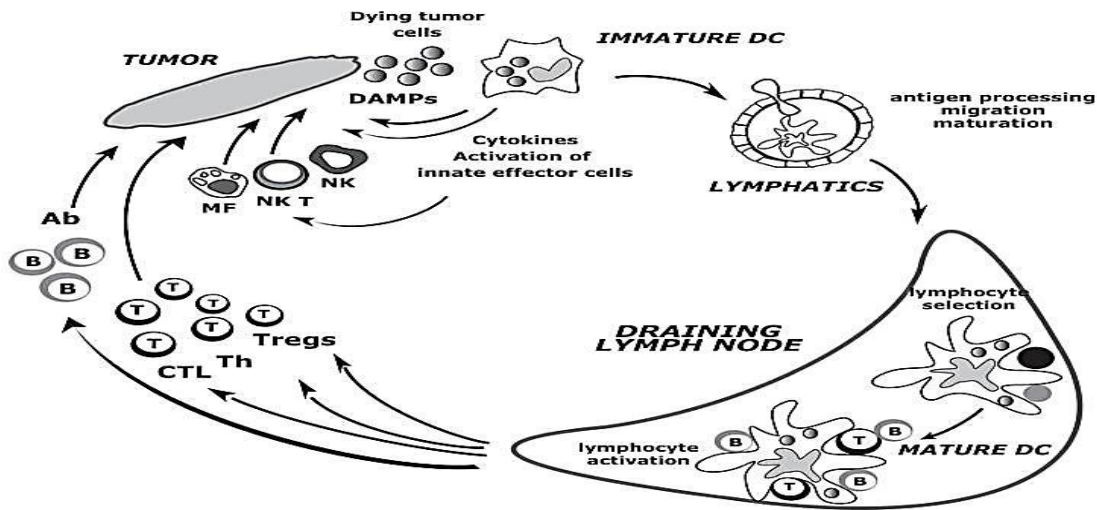


Fig. 3. Dendritic cells bind and process DAMs, which are then presented to cells of both innate and adaptive immunity, thus eliciting a potent anti-tumour immune response. (Taken from Dhodapkar et al. 2008)

4.3 Direct Immune Responses to Cancer

In patients with multiple myeloma, the latent period that precedes clinical manifestation of the disease - known as monoclonal gammopathy of undetermined significance (MGUS) period – has been shown to be characterized by strong T-cell mediated immune responses against autologous pre-malignant cells, suggesting that bone marrow-derived T-cells are capable of inhibiting the development of myeloma during its early stages. This T-cell response was however observed to become non-detectable, as the disease progresses from pre-malignant to end stage disease state [31]. Also, high CD8+ T-cell levels have been shown to be associated with increased survival rate in patients suffering from melanoma, when compared with melanoma patients having low levels of CD8+ cells.

The concept of immune surveillance is also evidenced by the occurrence of Para neoplastic syndromes, characterized by symptoms arising due to the presence of cancer but not from the cancer itself. These symptoms are largely caused by strong immune responses against a developing tumour and include cancer cachexia, hypercalcemia, Cushing syndrome and Trousseau syndrome [31].

Furthermore, dendritic cells (DCs) are known to invade different cancers and bind several damage associated molecules (DAMs) including Matzinger's damage signal molecules such as

HMGB1, heat shock proteins and uric acid, which they present to cells of both innate and adaptive immunity thus eliciting an extensive anti-tumour immune response. In the peripheral tissues, DCs bearing several antigens, including tumour-specific antigens, travel through lymphatic vessels and drain into adjoining lymph nodes where they present processed antigens to T-cells, using MHC class 1 and MHC class 11 molecules, as well as CD1 antigen presenting molecules, leading to T-cell mediated killing of cancer cells [32].

5. TUMOUR MARKERS AND IMMUNOGENIC CANCER CELL DEATH

Although extensive research has been done on the effector molecules that mediate immune surveillance of cancer, a comparatively less effort has been aimed at identifying the antigens and molecules present on the surfaces of cancer cells that activate the immune response against cancers. Previous studies in this area have identified molecules such as survivin, parathyroid hormone-related protein (PTHrP), human telomerase reverse transcriptase (hTERT), N-cadherin, erythropoietin-producing hepatocellular receptor tyrosine kinase class A2 (Eph A2) and synovial sarcoma X chromosome break point (SSX) proteins [33]. Others include alpha fetoprotein (AFP), epithelial tumour antigen, tyrosinase and melanoma-associated antigen (MAGE).

Apart from these tumour-associated antigens, cancer cells must also express Matzinger's danger signals such as heat shock proteins, chromatin associated protein, high mobility group box 1 (HMGB1), IFN- α , uric acid and some endogenous nucleic acids. This is usually followed by expression of a third group of molecules – the 'eat me' signals – and suppression of 'don't eat me' signals such as CD31 and CD47, resulting in the uptake of tumour cells by immature dendritic cells [34].

Cancer cells die by different mechanisms, including apoptosis and necrosis, depending on the stimulus. Autophagy, mitotic catastrophe and cellular senescence which can be triggered by cellular stress or malignant transformation, are also known to be involved in cancer cell death during the early stages of apoptosis. The processes that ultimately lead to clearance of such dead or dying apoptotic cells have been classified into four distinct stages including the release of 'find me' signals, expression of 'eat me' signals, processing of ingested cells and finally, degradation of processed cells, also referred to as post-engulfment consequences [35].

Several 'find me' signals have been reported in mammals including lysophosphatidylcholine, fractalkine, nucleotide ATP and uridine 5' triphosphate as well as sphingosine 1-phosphate (S1P). These molecules are involved in attracting monocytes to sites of apoptosis [35]. On arrival at the site of dying cancer cells, attracted

phagocytes must be able to distinguish damaged and dying cells, as well as dead cells from adjacent healthy cells. Recognition of such apoptotic cells is dependent on their surface expression of 'eat me' signals such as calreticulin (CRT) and phosphatidylserine, as well as changes in the charge and glycosylation patterns and alterations of ICAM-1 epitopes on cell surfaces. Studies in the past have shown that some chemotherapeutic agents such as anthracyclins could mediate a rapid pre-apoptotic translocation of calreticulin, an 'eat me' signal, from the lumen of endoplasmic reticulum, to the surfaces of cancer cells. Surface expression of calreticulin was also observed to attract phagocytes to cancer cells, thereby stimulating an anti-cancer immune response [29]. These observations suggest that combining the immune-enhancing effects of medicinal plants with conventional chemotherapy could produce a synergistic anti-cancer immune response capable of preventing cancer recurrence.

A critical evaluation of the evidences put forward in support of immune surveillance in man reveals that cancer cells take advantage of a weakened immune system to flourish. This therefore implies that boosting the immune system to function optimally could be a viable first step in the management of cancers. The immune system must be able to quickly recognize and clear dying or dead cancer cells expressing the relevant 'eat me' signals such as phosphatidylserine and calreticulin.

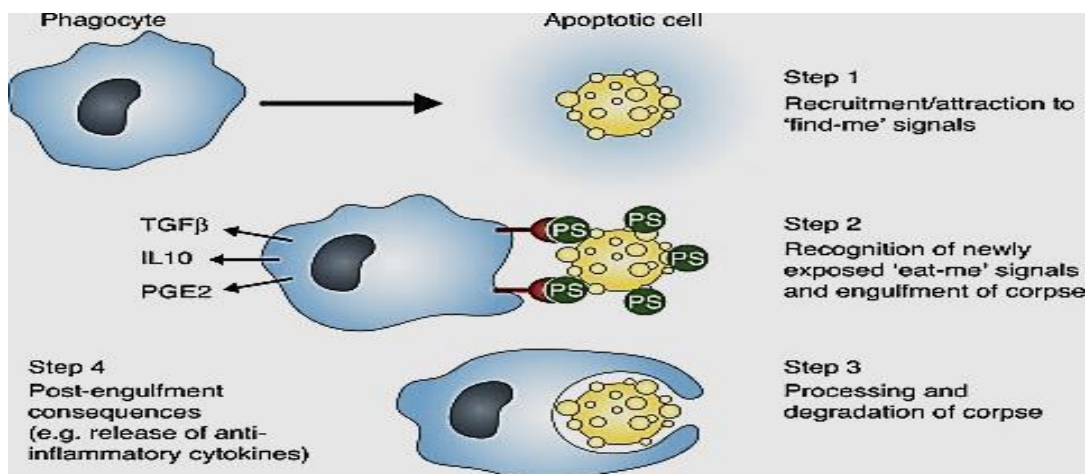


Fig. 4. 'Find me' signals such as lysophosphatidylcholine, fractalkine and nucleotide ATP, and 'eat me' signals such as phosphatidylserine and calreticulin are involved in the attraction, recognition and engulfment of cancer cells by monocytes and macrophages, while post-engulfment 'processing and degradation' facilitates the clearance of dying and dead apoptotic cells. (Taken from Ravi C, 2010)

Critics of the concept of cancer immune surveillance might once again question the development of cancers in individuals with apparently competent immune responses. However, the components of what constitutes competent and incompetent immune systems remain largely undefined. As rightly observed by Dunn and colleagues, even nude mice still possess some functional levels of adaptive immunity. Similarly, considering the huge number of molecules and cells involved in the regulation of anticancer immune responses, defects in one key molecule or class of cells like the APCs, even in the presence of optimal levels of other molecules and cells, could alter the supposed competence of an anticancer immune response in apparently healthy individuals.

Having established the role of a competent immune system in the elimination of cancer cells, including its mechanisms of surveillance and detection of tumour markers expressed on the surfaces of cancer cells, we proceed to highlight the potential role of herbal medicines in activating very potent anti-cancer immune responses. Highlighting the huge potentials of herbs in enhancing anti-cancer immune responses is important for several reasons. Standard treatment protocol for cancer usually involves surgical resection, chemotherapy and radiotherapy. While surgical removal eliminates the primary tumour and reduces tumour load, radiotherapy and chemotherapy target metastatic tumour cells around primary tumour and at distant parts of the body. Even with standard treatment, initial positive clinical responses are often followed by recurrence of malignancy, several months or years later. Current efforts are therefore aimed at eliminating the residual cancer cells, by stimulating the body's immune responses to fight against metastasised cells that are mainly responsible for cancer relapse [36]. Also, sensitivity of tumours to radiotherapy and chemotherapy has been observed to be profoundly influenced by the level of competence of the host's immune responses.

This synergy between conventional cancer treatment modalities and the immune system is largely embedded within the concept of 'immunogenic cancer cell death', whereby chemotherapy and radiotherapy-induced cancer cell death functions to stimulate natural anti-cancer immune responses, through the release and regulation of 'find me' and 'eat me signals.' The initiation and maintenance of such immunogenic cancer cell death is therefore

currently seen as one of the major aims of cancer chemotherapy and radiotherapy. Optimizing the immune responses of cancer patients during radiotherapy and chemotherapy through maximum utilization of the immune-boosting effects of natural herbs is therefore one of the aims of this review.

6. MEDICINAL PLANTS AND ANTI-CANCER IMMUNE RESPONSES

The World Health Organization (WHO) had reported that about 11% of all basic and essential medicines originate from plants. Drugs like digoxin, quinine, vincristine, atropine and morphine, originate from *Digitalis sp.*, *Cinchona spp.*, *Catharanthus roseus*, *Atropa belladonna* and *Papaver somniferu*, respectively. The WHO also estimates that about 80% of the world's population depends on medicinal plants for their health needs because of their preventive, curative, therapeutic and immune-modulatory effects [37].

The discovery of vinca alkaloids (vinblastine and vincristine) and isolation of cytotoxic podophyllotoxins in the 1950s directed the attention of researchers to medicinal plants as potential sources of anticancer drugs [38]. Vinblastine (VLB) and vincristine (VCR) from *Catharanthus roseus* (*Apocynaceae*) were the first anticancer agents introduced for clinical use [39]. Semi-synthetic analogues of vinca alkaloids such as vinorelbine (VRLB) and Vindesine (VDS) are currently used for the treatment of leukaemia, breast cancer, lung cancer, testicular cancer and Kaposi's sarcoma.

Other plant-derived anticancer agents that have found their way into the clinics include the Taxanes, Camptothecin derivatives and Homoharringtonine. An example of a taxane is paclitaxel or taxol, which was first isolated from the bark of the plant, *Taxus brevifolia Nutt.* (*Taxaceae*) and is currently used for the treatment of breast, ovarian and non-small-cell lung cancers. Camptothecin is derived from a Chinese ornamental tree called *Camptotheca acumata Decne* (*Nyssaceae*). Derivatives of camptothecin such as topotecan and irinotecan are currently used for the treatment of ovarian and colorectal cancers respectively [39]. Furthermore, plant based compounds such as allicin from *Allium sativum* (*garlic, lasun*); flavonoids and labdane diterpenoids from *Andrographis paniculata*; acetogenins from *Annona muricata* (*Graviola*) and tubeimoside-V from *Bolbostemma paniculatum*, among others,

have all been observed to possess some anticancer effects, either in vivo or in vitro.

Similarly, plants such as *Viscum album*, *Asparagus racemos*, *Panax ginseng* and *Tinospora cordifolia* are known to have strong effects on immune responses [40]. Studies by Kulkarni et al. in 2010 also revealed that the Avocado fruit has strong beneficial effects on immune responses, together with some minor cytotoxic effects [41]. Boosting the immune system with extracts from medicinal plants will enhance its capacity to respond to chemotherapy and radiotherapy-induced killing of cancer cells by promptly mopping up such dying or dead cancer cells, expressing the features of apoptosis. Continuous activation of anti-cancer immune responses with extracts from natural herbs will further enhance the immune system's potential of recognizing and responding to dying and dead cancer cells elsewhere, including distant and residual cancer cells, even after cessation of chemotherapy, thereby preventing cancer recurrence. Indeed, several studies have reported remarkable and specific anti-cancer immune effects of some medicinal plants.

6.1 *Tinospora cordifolia* (Giloy)

Tinospora cordifolia (Giloy) plant of the *Menispermaceae* family contains alkaloids, diterpenoids, flavonoids, berberine and lignins, which have been shown to mediate anti-neoplastic activity in cultured Hela cells. The active components of *T. cordifolia* have also been observed to be effective in the prevention of cyclophosphamide induced immunosuppression in mice and could potentially have a synergistic effect with cyclophosphamide in the treatment of cancer [42]. In a study to investigate whether the alcoholic extract of *Tinospora cordifolia* (ALTC) could activate the tumour-associated macrophages (TAM) of Dalton's lymphoma, Singh and colleagues observed that intraperitoneal administration of ALTC in Dalton lymphoma – bearing mice could augment the basic functions of macrophages such as phagocytosis, antigen presentation and secretion of IL-1, TNF and RNI. They also observed that ALTC could directly or indirectly destabilize the membrane integrity of Dalton's lymphoma. These findings suggest that apart from directly destroying Dalton's lymphoma cells and facilitating the release of apoptotic signals, intraperitoneal administration of ALTC could also activate macrophage mediated anti-cancer immune responses that could potentially slow down the growth and proliferation of tumours.

6.2 *Withania somnifera* (Ashwagandha)

The medicinal plant, *Withania somnifera* (Ashwagandha), commonly referred to as Winter cherry or Indian ginseng, have also been shown to possess very potent immuno-modulatory [43], antitumor and cytoprotective effects [44]. Apart from preventing cyclophosphamide, azathioprine and prednisolone induced myelosuppression, extracts from *Withania somnifera* have also been shown to be effective in the mobilization and activation of macrophage mediated phagocytosis of cancer cells. The anti-cancer properties of *W. somnifera* are believed to result from its ability to inhibit the expression of nuclear factor kappa B (NF- κ B), while potentiating apoptotic signalling in cancer cell lines. The anti-cancer activity of Indian ginseng against breast and colon cancers has also been observed to be stronger than that of doxorubicin [45].

6.3 *Allium sativum* (Garlic)

Another popular medicinal plant with strong anti-cancer immune effect is *Allium sativum* (Garlic), of the *Liliaceae* family. This medicinal plant contains allin, allicin and allyl sulphide, which are well known anti-cancer compounds involved in the inhibition of cancer proliferation. The active components of garlic have been observed to be effective in maintaining immune function homeostasis by enhancing production of natural killer (NK) cells and interleukin 10 (IL-10), while inhibiting production of pro-inflammatory cytokines (IL-1, IL-6, IL-8 and TNF) [46]. Extracts from *A. sativum* also enhanced production of IL-2 and augmented macrophage and T-lymphocyte activities. By inhibiting the production of pro-inflammatory cytokines, extracts from *A. sativum* prevents the development of chronic inflammation and ensures the establishment and maintenance of a microenvironment that is unfavourable for the initiation, progression and promotion of tumours.

6.4 *Boerhaavia diffusa*

The ethanolic extract of the medicinal plant *Boerhaavia diffusa* has been observed to significantly inhibit cell proliferation, while root extracts from the plant inhibited nitric oxide production in mouse macrophage cells, interleukin - 2 and tumour necrosis factor α in human peripheral blood mononuclear cells (PBMCs). These extracts however, did not affect intra cytoplasmic interferon gamma (IFN – γ) and cell surface markers such as CD 16, CD 25 and HLA – DR. This suggests that extracts from *B.*

diffusa could promote recognition of cancer cells bearing the so called 'find me' and 'eat me' signals by macrophages, while limiting the cytotoxic effects of reactive species and chemotherapeutic agents on normal cells. A number of other potential complementary and synergistic effects of medicinal plant extracts on conventional anti-cancer chemotherapy have been observed and published, including enhancement of immune responses by a polyclonal B-cell mitogen found in crude extracts of *Tinospora cordifolia*.

Apart from the popular medicinal plants such as *Catharanthus roseus* (Apocynaceae), *Allium sativum* (Garlic) and many others whose extracts have found their way to the clinics for treatment of cancers, there are many other medicinal plants that are still being investigated for potential anticancer and immune stimulatory activities, with very promising results. Examples of such plants include *Euphorbia hirta* Linn, *Gloriosa superba*, *Phyllanthus amarus*, *Eclipta alba*, *Cyclea peltata*, *Clitoria ternatea* Linn, *Centella asiatica*, *Biophytum sensitivum*, *Cassia fistula* L, *Scutellaria baicalensis*, *Urtica membranacea*, *Artemesia monosperma*, *Origanum dayi post* – to mention a few. The percentage of anticancer agents developed from natural products, especially medicinal plants, is expected to increase significantly above the current 60% in the next decade, if the level of attention being given to these medicinal plants with anticancer activity, is sustained and improved upon.

For example, results from studies on flavopiridol (isolated from *Dysoxylum binectariferum*) and meisoindigo (isolated from *Indigofera tinctoria*) have suggested very potent anticancer effects, with extremely minimal side effects, when compared to conventional synthetic anticancer agents and both agents are currently under different stages of clinical trials. [47]. Similarly, extracts from *Urtica membranacea*, *Artemesia monosperma* and *Origanum dayi post* have been observed to possess both dose and time dependent anticancer activity against human cancer cell lines, including haematological cell lines. These extracts were also observed to have little or no effect on healthy human cells. [48]. Furthermore, Adrienne and colleagues investigated the anti-malignant effects of flavonoid-rich ethanol extracts of mature roots of *Scutellaria baicalensis* on primary and recurrent glioma cell lines. Findings from their studies suggest a strong anti-proliferative activity against recurrent and drug resistant human glioma cell lines. [49].

Apart from conventional herbs, medicinal mushrooms are also important sources of natural products with both anticancer and immune modulatory activities. Many substances of mushroom origin, including polysaccharides, proteins, terpenoids, phenols, steroids, dietary fibres and polysaccharide-protein complexes, have been observed to possess both anticancer and immunotherapeutic effects in cancer patients. [24]. For example, in a study done to investigate the substances responsible for anticancer activity of mushrooms, Mohammad-Fata and Ghorban-Ali, 2007, observed that the β -D-glucan derivatives and fungal immunomodulating proteins (Fips) are largely responsible for the enhancement of effector immune cells, including components of both innate and adaptive immunity such as lymphocytes, macrophages, T-cells, dendritic cells and natural killer cells. [26].

Apart from modulating the activities of innate and adaptive immune cells, mushroom products such as lectins have also been observed to recognize and bind to abnormal glycans present on the surfaces of cancer cells. Such lectin-glycan interactions are known to induce apoptosis in cancer cells, leading to their recognition and consequent clearance by macrophages and other cells of the innate immune system [50].

7. CONCLUSION

This review systematically highlights the concept of immune surveillance of cancer cells from a historical perspective and links this concept with the principle of immunogenic cancer cell death, which is currently seen as the aim of conventional chemotherapy and radiotherapy. It further highlights the general and specific anticancer immune modulatory effects of some selected medicinal plants and their potential role in the current concept of immunogenic cancer cell death, during and after conventional cancer treatment. It clearly presents evidences in support of a potential role of medicinal plants in initiating and maintaining anticancer immune responses during and after chemotherapy, while ameliorating some of the unwanted side effects of chemotherapy, such as myelo-suppression and immunosuppression.

A thorough appraisal of reported anti-cancer effects of medicinal plants would reveal that the potential benefits of herbal remedies in the treatment of cancers have been grossly underutilized. Conscious efforts must therefore be made, to highlight these benefits and bring

the active immune-enhancing components of medicinal plants into the mainstream of cancer treatment protocols. However, despite these amazing potentials, greater effort and commitment is still needed, to optimize current methods of extraction, purification and molecular characterization of the active components of medicinal plants that are responsible for their anti-cancer immune effects. This will not only improve public trust and confidence in the use of immune-boosting medicinal plant extracts in cancer treatment but also effectively reduce likely side effects and toxicity, even though herbal remedies are widely known to be less toxic, with little or no side effects, when compared with synthetic drugs.

A gradual but well planned scheme of integration that would involve massive literature review, data analysis, sample collection, improved research and validation is all that is needed, to fully harness the anti-cancer immune effects of medicinal plants for the benefit of cancer patients. If fully utilized, the anticancer immune activities of medicinal plant extracts could prove to be the antidote to cancer recurrence, which has remained a major challenge to conventional treatment modalities.

If the initiation and maintenance of immunogenic cancer cell death is truly a major aim of cancer chemotherapy, then pharmaceutical companies and research institutions must begin to embrace medicinal plants and phytotherapy as vital components of post-surgical anticancer therapy. They must also be prepared to encourage and invest in large scale research activities into the potential anticancer immune effects of popular medicinal plants, whose immune modulating activities could be all that is needed to abate cancer recurrence. Doctors, pharmacists, nurses and other healthcare providers must also be encouraged to be involved in active research and promotion of use of medicinal plant extracts as complementary anticancer and immune-boosting agents during and after conventional cancer treatment. Well-structured enlightenment programmes in the form of seminars, conferences and public health campaigns could be organised at local and international levels to bring laboratory research findings on medicinal plants' anti-cancer immune properties closer to the populace. This will go a long way in demystifying the amazing anticancer immune effects that have been observed in many medicinal plant extracts and help in promoting their acceptance as potent and safe anticancer agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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