TITLE:

Escape from immunotherapy: possible mechanisms that influence tumor regression / progression.

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Abstract

Tumor escape is one major obstacle that has to be addressed prior to designing and delivering successful immunotherapy. There is compelling evidence to support the notion that immunogenic tumors, in murine models and cancer patients, can be rejected by the immune system under optimum conditions for activating adaptive and non-adaptive anti-tumor immune responses. Despite this capability, a large number of tumors continue to grow and evade recognition and/or destruction by the immune system. The limited success in current immunotherapeutic strategies may be due to a variety of reasons: failure of effector cells to compete with the growing tumor burden, production of humoral factors by tumors that locally block cytotoxicity, antigen / MHC loss, T-cell dysfunction, production of suppressor T – cells to name but a few or therapy ineffectiveness for the particular malignancy being treated. To optimise immunotherapy strategies correction of immune activating signals, eradication of inhibitory factors and the evasion from newly developed immunoresistant tumor phenotypes need to be simultaneously considered.

Introduction

The immune defence mechanisms are the least effective and the final barrier in the natural mechanisms against cancer formation (carcinogenesis) (49). Both innate and adaptive immunity induce anti-tumor effects via the activity of NK cells, NKT cells, macrophages, neutrophils, eosinophils, various cytokines and specific CTLs to name but a few. However spontaneous regression is rarely observed in malignant tumors, hence the need for immunotherapeutic intervention is required in order to eradicate the tumor.

Advances in both molecular and cellular immunology have improved our understanding of tumor-host interactions, although much still remains elusive despite the progress made in identifying a large number of tumor antigens and new immunotherapeutic strategies. The majority of tumors manage to “escape recognition” and currently there are a number of mechanisms known for tumor escape which have been described – loss / downregulation of HLA Class I, downregulation, mutation or loss of tumor antigens, alterations in cell death receptor signalling, production of immunosuppressive cytokines and suppressor T-cells and the involvement of

indoleamine 2,3-dioxygenase in suppression - all of which will be discussed in detail later.

It is believed that a T-cell response does exist but is ineffective as the effector phase of the immune response is inadequate. This notion may explain why current immunotherapeutic strategies have limited success. From the immunological perspective efficient therapeutic intervention should focus on boosting existing anti-tumor responses and to sustain large numbers of effector T cells at the tumor site.

Recently, this was clearly demonstrated in melanoma patient where T cell responses to vaccines occurred primarily in the presence of pre-activated T cells prior to vaccination (111). Analyses of cell mediated immunity against defined melanoma antigens using tetramer staining and an IFN-γ secretion assay suggested that specific T cell responses often exist during tumor progression (99). Adjuvants such as cytokines have been used when immunising with peptides and proteins (48,54,100,118). Immunisation with DNA vaccines leads to the expression of tumor antigens and their processing by antigen presenting cells (APCs) (112), whereas the direct delivery of antigens as proteins by cell fusion or cDNA to dendritic cells (DCs) represents a more direct attempt to generate antitumor responses (2).

Some anti-tumor therapeutic interventions have met with limited success which has been attributed mainly to the "tumor – escape" phenomena i.e. tumor cells have the ability to evade immune recognition and/or destruction, causing major obstacles in using this modality for the treatment of cancer. This was clearly demonstrated in prostate tumor (TRAMP-C1) model where the injection of Fms-like tyrosine kinase-3 ligand (flt3-L) induced short-term tumor stabilization and regression followed by tumor relapse (21). Failure of therapy in this model revealed several important immunosuppressive characteristics of the prostate tumor microenvironment including the down regulation of MHC class II in DCs, profound deficiency in the expression of CD3ε (CD3epsilon) and the beta chain of the T cell receptor (TCR) of tumor-associated T cells. Cancer cells can be detected and destroyed by cytotoxic T lymphocytes (CTLs) in many experimental models and human tumors but on diagnosis the metastasis of many human tumors can be shown to correlate with unresponsive T cells. Progressive tumor growth occurs, in part, because the cancer cell is capable of escaping immune recognition and not because the immune system is defective (16). CML patients for example possess CTLs detectable by tetramer staining and

cytotoxicity that recognise HLA-A3 restricted peptide, although the number of CTLs may be too few to mediate complete rejection of the cancer (23).

Deletion or suppression of activated CTLs can occur as well as their migration away from the tumor site. The precursor frequency can be too low, such that the effector cells fail to compete with the growing tumor burden. However, it is equally possible that the therapies are ineffective for the particular malignancy being treated. In order to fully appreciate the role of tumor escape in the failure of immunotherapy, an understanding of the basic principles of immunosurveillance and its role in determining tumor phenotypes is necessary.

The hypothesis first proposed by Thomas & Burnet in the 1970s implied that the immune response to tumor occurred at an early stage in tumor development – "eradicating the cancer before it became apparent" (28,87).

Many studies have evolved in support of this theory and evidence exists to suggest that in some models and under appropriate conditions, immunosurveillance can play an active role in suppressing the growth of early tumors. Hence in this paradigm, when tumors do successfully grow, they are thought to have escaped from surveillance and a number of reviews have been written to support this theory (56,78,97).

The following will focus on the multiple mechanisms that exist leading to "tumor-escape" from the immune system; these factors have to be taken into consideration when designing novel therapeutic strategies. The aim of this review is to highlight the required mechanisms of tumor escape and how they influence the failure of immunotherapy.

Activation versus Suppression during Tumor Progression.

Escape from immune-surveillance is a major mechanism for tumors to grow progressively. Tumors may grow undetected by the immune system, being seen as "normal tissue" hence benefiting from any mechanism that makes them appear "healthy" and less dangerous i.e. exhibiting no danger signals for immune activation (56,86). It is believed that even during progressive growth the tumor has the ability to activate the immune system, and that a fine balance between activation and suppression exists, which determines the fate of the tumor. Tumor vaccines may induce activation and expansion of specific $CD8^+$ T cells and destruction of tumor cells in cancer patients; this was observed in approximately 5-20% of vaccinated

melanoma patients. However, this activation can be fraught by the lack of appropriate co-stimulation or the presence of immunosuppressive cytokines such as IL-10 and TGF-β. The eventual fate of the tumor is therefore determined by the net effect of immune activation and inhibition. (56).

Killing of tumor cells *in situ* by suicide gene transfer to induce cell death through a nonapoptotic pathway was shown to be associated with enhanced immunogeneicity (74). Similar observations were also reported by our group where immunisation with irradiated RENCA cells (murine renal carcinoma) infected with DISC-HSV (Disabled Infectious Single Cycle – Herpes Simplex Virus) that enhanced immunity to tumor challenge with live parental tumor cells that induced cell death by necrosis as opposed to apoptosis (5) providing the immune system with additional activation as the cells were undergoing necrotic cell lysis enhanced tumor antigen processing and presentation by APCs and hence an increase in T cell activation (59,70).

The mechanisms of tumor escape from immune recognition / destruction are likely to be multifactorial including downregulation of MHC Class I molecules (4,52,96,131), loss of tumor antigens (26,44), defective death receptor signalling (24,47,73,116,117), lack of co-stimulation (104), production of immunosuppressive cytokines (85) and suppressive cells (12,35,61,72,113) as shown in figure 1.

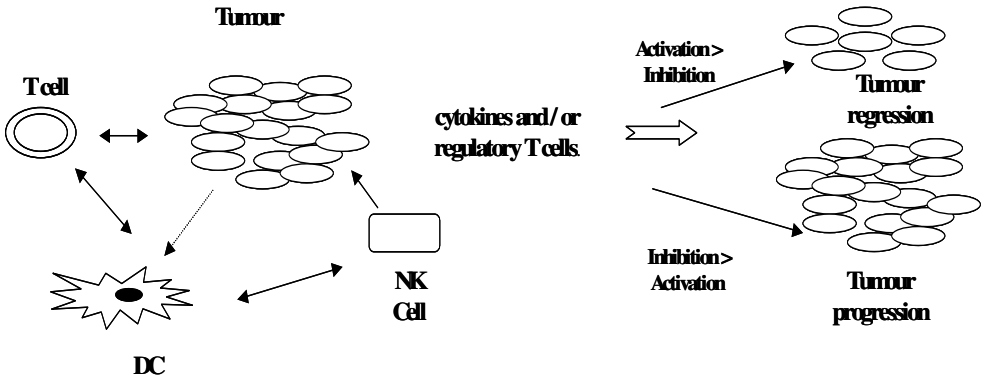

Figure 1:- A schematic diagram summarising the processes of activation and suppression during tumor progression.

Involvement of Indoleamine 2,3 –dioxygenase in immunosuppression.

The mechanisms that mediate immune tolerance to cancer are not well understood, but recent findings have implicated the tryptophan/IDO (indoleamine 2,3-dioxygenase) metabolic pathway as one of many mechanisms involved. IDO is an enzyme ubiquitously distributed in mammalian tissues and cells converting tryptophane to N-formylkynurenine (34). This cytosolic enzyme catalyses the initial and rate limiting step in the catabolism of tryptophan in the kynurenine pathway (76). Low levels of tryptophan at the tumor site causes T cells to arrest in the G1 phase of the cell cycle (125). Initially the enzyme was recognised for its anti-microbial activity by allowing cells to deplete tryptophan from intracellular pool or local microenvironment (114), however recently, IDO was reported to be constitutively expressed by most human tumors and its expression by immunogenic mouse tumor cells prevents their rejection in pre-immunised mice, correlating with the lack of specific T cell accumulation at the tumor site (125).

IDO expression is an inducible feature of splenic DC subsets, and provides a potential explanation for their ability to regulate T cells. Induction of IDO completely blocked the clonal expansion of T cells from TCR transgenic mice following the administration of the immunomodulatory reagent CTLA4-Ig, whereas the same treatment did not block T cell clonal expansion in IDO-deficient recipients. Suppression of allogeneic T cell responses in vitro and in vivo by interstitial APC was also shown to be IDO-dependent and blockade of IDO activity with 1-MT (1-methyl-

DL-tryptophan (3)) or addition of exogenous tryptophan allowed the recovery of T cell proliferation in MLR assays (115). In humans, only a discrete subset of APCs that co-express the cell-surface markers CD123 and CCR6 was shown to express IDO and inhibit T cell proliferation *in vitro* (79). These results clearly demonstrated that the IDO pathway might represent a potential mechanism for DCs to regulate the immune response to tumor antigens.

Loss / Downregulation of HLA Class I.

Altered MHC class I antigen expression in tumors is a well known phenomena. The selective loss of MHC class I alleles was subsequently described in a variety of mouse models including the TL leukaemic cell line, MCA-induced tumors and murine leukaemia virus induced tumors. The altered phenotypes of MHC Class I antigen expression permits tumor cells to avoid recognition or survive attack by $CD8^+$ T-cells capable of mediating cytotoxicity. This phenomenon has been studied in a series of tumor samples by immunohistochemical techniques and has been shown to be widespread (58).

HLA Class I molecule downregulation occurs frequently in many cancers and this abnormality might adversely affect the clinical course of disease and the outcome of T cell based immunotherapies. Over the past few years HLA Class I expression has been characterised in human tumors. Changes in HLA Class I expression can occur through various mechanisms – namely, mutations in genes and abnormalities in the regulation and/or defects in HLA Class I dependent antigen processing. These mutations modulate the susceptibility of tumor cells to *in vitro* lysis by CTLs and natural killer (NK) cells. Immune selection of tumor resistant CTLs and/or NK cells may explain the rapid progression and poor prognosis of cancers exhibiting HLA Class I antigen downregulation (43).

To date a number of investigators have classified the types of HLA Class I loss into different phenotypes, and Table 1 summarises the HLA phenotypes that are recognised.

Table 1: *Major HLA phenotypes in tumors and the associated molecular mechanisms. (Adapted from Cabrera et al 2003 (16)).*

HLA Phenotype	Mechanisms	References
(I) HLA Class I Total Loss	β_2m mutations	37,55,88
	Alterations in antigen processing machinery	37,105
(II) HLA haplotype Loss	LOH in chromosome 6	31,37,91,92
(III) HLA Allelic Loss	Mutations of HLA Class I genes	13,37
(IV) HLA A, B, C locus downregulation	Alteration of transcriptional factors	37,39,110
(V) Compound phenotype	2 or more independent mechanisms	37,45,93

A number of studies have been carried out to assess HLA Class I expression in tumors. In bladder carcinomas, 72% of the tumors studied showed at least one alteration in HLA expression and these were classified into different phenotypes depending on the type of downregulation/loss (18); expression was correlated to the degree of differentiation and tumor recurrence. To determine the type of HLA loss occurring during tumor progression, tissue samples from breast, colorectal and laryngeal carcinomas were analysed (17). HLA-B44 allele loss was observed more frequently than any other HLA Class I allele – suggesting an important role for this allele in tumor escape.

Furthermore, mechanisms of MHC Class I loss were also investigated in colorectal carcinoma (15). It was shown that β_2m mutation and LMP7 / TAP2 downregulation were responsible for the total loss of MHC class I expression-which contribute to the failure of T cell recognition during the immune response.

In murine models, the B9 primary tumor clone (H-2 negative) and its metastatic colonies were studied in immunocompetent Balb/c mice (37). They showed that 83% of the metastasis obtained in different syngeneic Balb/c mice repeatedly exhibited a phenotype different from the original B9 clone. The alterations were identical in different colonies of different syngeneic Balb/c mice.

Variation in the MHC Class I expression following immunotherapy has been observed in a murine colon carcinoma model (Ahmad, Ali, and Rees, unpublished observation). The CT26 (colon carcinoma) murine tumor model was used to investigate the expression of MHC Class I antigens in mice failing to regress their

tumors following immunotherapy (progressor) and tumor bearer (no therapy) animals (6). The DISC-HSV/mGM-CSF vector used in this study is an attractive tool for the delivery of cytokines to the tumors (11,94,120). This vector was developed by genetically inactivating the HSV-2 for use as a vaccine, by the deletion of the essential glycoprotein H (gH) gene – restricting it to one cycle of replication (11). On infection, the virus would release non-infectious particles together with the transduced cytokine e.g. GM-CSF. In our model the DISC therapy was administered intratumorally to mice with established subcutaneous (s.c) tumors, and approximately 40% of the treated animals fail therapy and the tumors continue to grow progressively (3,6). MHC Class I ($H\text{-}2L^d$) expression was investigated in both tumor bearing and progressor mice. The results (Table 2) clearly demonstrated that a high proportion (55%) of tumors from progressor mice showed loss of class I MHC antigen, unlike the control tumors (treated with PBS) all of which expressed MHC I antigen. Interestingly, the tumor cells from the progressor animals re-expressed Class I following overnight *in vitro* culture, suggesting that the tumor micro-environment may be responsible for the transient downregulation of Class I expression as opposed to a mutation in the β2m gene (95) or a downregulation in LMP7 / TAP 2 as reported in other studies (15).

Table 2: *A total of 18 tumors were studied (11 progressors, 7 PBS controls) for the expression of* $H2L^d$*. The results have been combined for the immunohistochemistry and FACS analysis, such that 45% of the therapy failures completely lost* $H2L^d$ *surface expression whereas the tumor bearer animals retained a degree of expression (low, partial or full expression) (A.Talasila – MSc. Thesis, Nottingham Trent University, UK).*

Treatment	No Expression (%)	Low Expression (%)	Partial Expression (%)	Full Expression (%)
Tumor Bearer	0	29	29	42
DISC/mGM-CSF	45	28	27	0

Recently a number of studies have investigated the expression of Class II in patients. Antigen – presenting cells are crucial for the induction of an antigen – specific immune response and downregulation / loss of expression in cell surface molecules such as HLA Class II may contribute to an impaired immune response. This phenomena was observed in PBMs of melanoma patients (123). A number of

different carcinomas (breast, ovarian and prostate) have been investigated for the expression of MHC Class I and II antigens (62, 69, 107) where the absence / downregulation of these recognition molecules correlated with the tumor's ability to avoid immune surveillance and development of metastatic dissemination of the malignancy.

Down-regulation, mutation or Loss of Tumor Antigens.

Alterations in tumor associated antigen (TAA) expression is one of the mechanisms by which tumor cells can escape CTL detection. Modifications in the TAA expression can range from down-modulation to complete loss, and loss of antigen expression can occur independently of the deregulation of HLA Class I expression. Tumor antigen expression is known to be heterogeneous, even within the same tumor (56); and a decrease in the expression of gp100 and MART-1 was associated with disease progression (26,44). In spite of the presence of TAA-specific CTL, immunogenic tumors can eventually grow and kill the host in animal models. Tumor escape in the murine mastocytoma P815 tumor was shown to be due to the emergence of stable antigen-loss variants (124).

Antigenic drift, a mechanism used by viruses to escape immune recognition, has recently been described for tumors. Transgenic mice expressing TCR for a single antigenic epitope have been used extensively in establishing antigenic mutation(s) as a mechanism for viral escape of T cell recognition (22,98). A transgenic mouse line expressing TCR specific for tumor antigen P1A35-43 presented by H-2L^{d} was also developed and used to study tumor escape mechanisms. The recurrence of tumors in mice that have responded favourably to transgenic T cell adoptive therapy was found to correlate with the presence of tumor variants with mutations within the PIA epitope (9). These mutations severely diminish T cell recognition of the tumor antigen by a variety of mechanisms, including modulation of MHC: peptide interaction and TCR binding to MHC: peptide complex. In another study, using a SCID mouse model and MART-1/Melan-A-sepcific CD8^{+} T cell clone against autologuos melanoma, it was found that the *in vivo* immuno-selection of antigen-loss variants was dependent on the presence of sub optimal levels of antigen expression (66); and loss of the immunodominant T cell-defined MART-1/Melan-A antigen and downregulation of the TAP-1 gene has been identified in a recurrent metastatic melanoma. Restoring the "antigen loss" in the variant tumor cell line by simultaneously providing both the

MART-1/Melan-A gene (by retroviral transfer) and the TAP-1 gene (by a bioballistic approach) resulted in tumor cell sensitivity to MART-1/Melan-A-specific cytotoxic T lymphocytes suggesting antigen loss and downregulation of the peptide-transporter protein TAP-1 expression (68). The emergence of tumor escape variants is more likely to result after effective immunotherapies.

Alterations in cell death receptor signalling.

The expression of Fas Ligand (FasL), a well-known cell death receptor ligand, on tumor cells has been implicated in their evasion of immune-surveillance (20,24,90,116,117). Apoptosis mediated via CD95 (Fas/Apo-1) is a key regulator for the biology of normal and malignant cells. However, Fas surface expression does not necessarily render cells susceptible to FasL-induced death signals, suggesting a role for inhibitors of the apoptosis-signalling pathway. Phosphatidylinositol 3'-kinase (PI3 K) and Akt (protein kinase B) mediate the survival signal and allow cells to escape from apoptosis in various human cancers. PI3 K inhibitors, such as LY294002 inhibited cell proliferation and increase apoptosis in the human gastric carcinoma cell line by the down-regulation of Mcl-2 and phosphorylated Bad proteins, which are anti-apoptotic factors and belong to the Bcl-2 family (84). Acquisition of apoptosis resistance is a typical mechanism of chemotherapy failure in small-cell lung cancer, which was correlated, with the over-expression of Bcl-2 (32).

Defects in receptors/signalling can facilitate tumor cell survival and proliferation. The Fas/FasL complex forms and engages caspase-8 which autoactivates itself and cleaves caspase-3, -6 and –7 (101). The caspase-8 inhibitor, cellular FLICE-inhibitory protein (cFLIP) is expressed in many tumors making them resistant to death receptor mediated apoptosis (47). FLICE/caspase-8-inhibitory protein (cFLIP) is a recently identified intracellular inhibitor of caspase-8 activation that potently inhibits death signalling mediated by all known death receptors, including Fas, TNF-receptor (TNF-R), and TNF-related apoptosis-inducing ligand receptors (TRAIL-Rs), The increased expression of cFLIP in tumor cells is thought to contribute to immunoresistance to T cells *in vivo* (73) while the downregulation/loss of Fas expression resists apoptosis. In patients with B-cell chronic lymphocytic leukemia (B-CLL cells), an increase in cell-surface CD95 expression on T cells was associated with reduced progression-free probability and poorer survival (40). In TRAIL-mediated apoptosis, the loss of

expression of all TRAIL receptors can occur by many mechanisms including the loss of caspase-8 by chromosomal loss or mutation (42).

Cells expressing low levels of Fas (Fas^{low}) in the B16F10 tumor model grew more slowly in comparison to the cells expressing high levels of Fas. Fas^{low}-cells when injected showed enhanced tumor growth in mice depleted of neutrophils, suggesting that inactivation of neutrophils was an important mechanism by which tumors could escape destruction (66). The expression of FasL has also been studied in cervix adenocarcinomas, where FasL appears to play an important role in immune evasion, progression and metastases of the tumor (53). In contrast, the intracellular expression of FasL in breast cancer and normal tissue, as determined by immunohistochemistry, is unlikely to be an important marker for immune evasion (90). The expression of membrane bound FasL (mFasL) on colon cancer cells as a potential mechanism to inhibit host immune function by inducing apoptosis of host lymphocytes was investigated (109). mFasL can be cleaved to release a soluble FasL (sFasL) to spread the apoptosis induction effect within the environment. These findings have suggested sFasL as a mechanism by which tumor cells can avoid immune attack.

Immunosuppressive Cytokines.

Tumor cells produce a number of cytokines and chemokines that can have a suppressive effect on immune cells. In non-small cell lung cancer patients, the mRNA expression of IL-4, IL-10, TGF-alpha, and TGF-beta1 was significantly higher than that of IL-2, IL-12, IL-18 and INF-gamma as determined in pleural effusion and tumor tissue (64). This predominant expression of type II (immunosuppressive) cytokines mirrors an immunosuppressive state in the immunological microenvironment. Vascular endothelial growth factor (VEGF) is secreted by many tumors (121), and in the past few years VEGF has been required as a contributory factor in tumor escape. VEGF is not only important for tumor vascularisation but is also a key factor produced by solid tumors to inhibit immune recognition (82), and prevent DC differentiation and maturation by suppressing the transcription factor NF-κB in haemotopoietic stem cells (85). Blocking NF-κB activation in haemotopoietic cells by tumor-derived factors is thought to be a mechanism by which tumor cells can directly down-regulate the ability of the immune system to generate an antitumor

response (85). Elevated VEGF blood concentrations have been correlated with poor prognosis in human neoplasms (65), possibly as a result of its angiogenic properties and/or its ability to suppress DC maturation (46,65). VEGF was negatively related to DC infilteration in immunohistochemical studies on resected lung cancer samples (46) and activation status of DC and the concentration of VEGF in the peripheral blood have been shown to reflect the malignancy of NSCLC (30).

Supernatants collected from tumor cells of AML patients were shown to inhibit T cell activation, Th1 cytokine production and to prevent activated T cells from entering the cell cycle (14), however, no TGF-β, IL-10 or VEGF were detected. The T cell immunosuppression induced by AML cells provides a mechanism by which leukemic clones could evade T cell mediated lysis by inhibiting the NF-κB, c-myc and pRb pathways (14). The production of soluble factors such as VEGF, IL-10, TNF and TGF-β is a proposed mechanism for tumor cells to avoid immune recognition, and the effects of these factors appear to be two-fold: to inhibit a) the effector function and b) the development of the immune cells by acting in the early stages of immunopoiesis (82).

IL-10 is a cytokine often quoted as being suppressive and high levels of IL-10 have been reported in patients with melanoma (33) and pancreatic cancer (10,77). IL-10 has the ability to exert its effects in many ways:- it inhibits antigen presentation, IL-12 production and the induction of Th1 responses in vivo (25,106).

High concentrations of TGF-β are also found in cancer patients (122) and are usually associated with "tumor-progression" (38) and poor responses to immunotherapy (27). The levels of TGF-β are higher in patients with disseminated melanoma when compared to those with localised disease (60). TGF-β has also been shown to induce the overproduction of IL-10 in tumors, leading to immunosuppression of antitumor responses. This suppression was reversed and Th1 responses reconstituted on the administration of anti-TGF-β antibodies in vivo (67).

Alterations in the expression of signal transduction molecules

Many different mechanisms may contribute to immune evasion. In pancreatic cancer, the loss of signal transducing CD3 zeta chain (CD3ξ) of TILs has been attributed to immune escape together with the production of immunosuppressive cytokines and

local impairment of TILs (126); CD3 zeta loss in many cases has also been correlated with elevated levels of IL-10 and TGF-β. The loss of the CD3ξ chain has been studied extensively and has been proposed as a mechanism by which tumors are able to escape. The zeta chain is present as a large intracytoplasmic homodimer in the TCR forming part of the TCR-CD3 complex. It functions as a single transducer upon antigen binding. (50). Hence any alterations in this chain could result in changes in the signalling pathway. In pancreatic cancer, the loss of signal transducing CD3ξ chain of tumor infilterating lymphocytes (TILs) has also been attributed to immune escape together with the production of immunosuppressive cytokines and local inactivation of TILs (126). Alterations in the expression of CD3ξ chain has also been correlated with elevated levels of IL-10 alone or in association with the downregulation of IFN-γ (81). A general downregulation / decreased expression the CD3ξ chain has been reported in tumors such as cervical and colorectal carcinomas (57,80,103). In cervical cancer the reduced expression of the zeta chain was associated with a reduction in cellular functions such as the production of TNF (57). In contrast, PBMCs from breast cancer and colorectal carcinomas patients do not show a decrease in TCR zeta expression yet the former exhibit an impairment in T cell function (81).

Lack of co-stimulation

Most tumors do not regress and continue to grow in spite of the presence of spontaneous or antigen (vaccine)-induced immune responses. The existence of systemic immune responses may not by itself be sufficient to cope with the complex nature of tumor-host interactions because of factors: insufficient co-stimulation to induce T-cell response may further contribute to the lack of effective immunity. There are a number of molecules that normally perform co-stimulatory functions by interacting with their counter ligand/receptor on T cells to provide the critical second signal for T-cell and/or APC activation including: CD80 (B7-1) / CD86 (B7-2) binding to CD28 and CTLA4 respectively; CD40L on activated CD4 helper cells binding to CD40 receptors on APCs; human CD58 and mouse CD48 (LFA-3) binding to CD2; and CD54 (ICAM-1) binding to LFA-1. Members of the TNFR super-family including CD27, CD30, 4-1BB and OX40 have also been shown to transmit a co-stimulation signals to leukocytes (41,108).

It is a recognised phenomenon that T cells are rendered anergic due to the lack of co-stimulatory molecule(s) expression by tumor cells (104). Tumor cells are able to induce antigen-specific tolerance or anergy on the basis of MHC-1-restricted antigen presentation without the expression of co-stimulatory ligand(s) (1). This unresponsiveness however can be reversed when tumor cells are genetically modified to express co-stimulation molecules. Chen et al (19) showed that the insertion of genes encoding B7.1 and /or B7.2 molecules into tumors generally increases their immunogenicity.

Recently, fusagene vectors were developed to encode multiple gene products as fusion proteins from a single cistron to increase the immunogenenicity of target tumor cells (36). Analysis of over 100 individual clones derived from human and murine tumor cell lines demonstrated efficient expression and biological activity of each of the proteins. Tricistronic viral vectors co-expressing IL-12 and B7.1 have been used in the immunotherapy of cancer (128); the vectors generated could be used in immunotherapy for the treatment of multiple myeloma and other cancers as they were shown to stimulate allogeneic mixed lymphocyte proliferation and provoke increases in CTL responses and IFN-γ release from normal donor lymphocytes exposed to the parental U266 cells.

In addition, co-stimulation through molecules like 4-1BB was also found to be critical in the expansion and differentiation of CTLs. Systemic administration of an agonistic mAb against 4-1BB enhanced the CD8 T-cell response, leading to the eradication of established AG104A sarcomas and P815 mastocytomas in vivo (75). However, resistance to this treatment modality by a number of poorly immunogenic tumors, including the TC-1 lung carcinoma and B16-F10 melanoma, was shown to be due to tumor antigen-specific CTLs “ignorance” rather than anergy or deletion; breaking CTL ignorance by peptide immunisation was necessary for the anti-4-1BB to function to enhance T-cell responses (129). Given the importance of co-stimulation in the regulation of immune responses against cancer, the manipulation of this pathway to increase immunity represents a promising therapeutic approach. Co-stimulation through OX40L for example would be advantageous, since its expression is primarily detected on recently stimulated antigen specific $CD4^+$ T cells (127, our unpublished data), which is considered advantageous for generating CD8+ T-cell responses and anti-tumor immunity.

Immunosuppressive cells

There are at least 2 types of cells that when produced can impose a suppressive effect on the host's immune system ($CD4^+$/ $CD25^+$ T cells and $Gr1^+$/ $CD11b^+$ myeloid cells), hence providing the tumor with the opportunity to escape immune recognition. Elucidating the mechanism(s) of T cell unresponsiveness in cancer is critical for the design and application of an effective cancer immunotherapy. Inhibition of a number of T lymphocyte functions in tumor-bearing hosts has been extensively investigated (63,89), and suppressor cells have been shown to play a role in the progression of cancer (29). $CD4^+$ $CD25^+$ T cells are known to be immunoregulatory and are important in immunological tolerance to self-antigens (72) and inhibition of T cell proliferation (119). They constitute approximately 5-10% of the $CD4^+$ T cells in both humans and rodents and their removal induces autoimmune diseases in various locations in the body. Sutmuller and colleagues (113) demonstrated that the depletion of $CD4^+$ $CD25^+$ T cells followed by an injection of an antibody which blocks CTLA-4 enhanced T-cell reactivity to a known tumor associated antigen. However, it is thought that the mere depletion or blocking of T regulatory cells is not sufficient to successfully treat established tumors (8).

The existence of anergic and functionally suppressive $CD4^+CD25^+$T cells was demonstrated in patients with melanoma undergoing immunisation with known melanoma antigens. The degree of inhibition of T cell proliferation was proportional to $CD4^+CD25^+$T cells present; the addition of IL-2 reversed their hypo-responsiveness and abrogated their suppressive function (51). An increase in CD4+CD25+ regulatory T cells was shown to be correlated with immunosuppression and tumor progression in patients with gastrointestinal malignancies (102). Increased numbers of $CD4^+CD25^+$T cells secreting TGF-β was detected in tumor infiltrates from patients with early and late-stage epithelial tumors (130). These observations provide clear evidence for the contribution of $CD4^+CD25^+$T cells to immune dysfunction in cancer patients. Immunotherapy aimed at decreasing the role of regulatory T-cells would be advantageous to successfully treating cancer.

In addition, immune suppression in tumor bearing mice has been attributed to the presence of cells with an immature myeloid phenotype that express the granulocyte – monocyte markers $Gr1^+$/$CD11b^+$ (12), and accumulate in the spleens and lymph nodes (71) and blood of tumor bearing mice (Ahmad, Ali and Rees, unpublished data). They

are capable of inhibiting antibody production, CTL generation, T cell function and lymphocytic proliferation (61,71). Accumulation of $Gr1^+/CD11b^+$ cells and their ability to inhibit the T cell function has been reported in cancer patients (7). The $Gr1^+$ cells are also able to decrease CD3ξ molecule expression significantly (83), and inhibit MHC Class I dependent antigen-specific $CD8^+$ T-cell (35).

Conclusion

This review has highlighted the main mechanisms by which the tumors are known to escape from immune recognition. Given the many different potential mechanisms that tumors can acquire to avoid or subvert adaptive immunity, future generation immunotherapeutic strategies will need to consider not only how to promote antigen driven T and B lymphocyte responses and their effective targeting to residual tumor, but also to understand how the mechanism(s) of tumor escape can be dealt with. Current research is exploring the application of combination therapy that utilises several treatment modalities, which may include sequential chemo-, radio- and immunotherapy protocols**.**

REFERENCES:

1.Abken H, Hombach A, Heuser C, Kronfeld K, Seliger B. (2002). Tuning tumor-specific T-cell activation: a matter of costimulation? Trends Immunol. 23(5):240-5.

2.Adams M, Jasani B, Colaco CS, Mason MD. (2000). Dendritic cell approaches to immunotherapy. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp 237.

3.Ahmad M, Rees RC, McArdle SEB, Li G, Mian S, Entwisle C, Loudon P, Ali SA.(2003). Regulation of CTL responses to a MHC- restricted class I peptide of the gp70 tumor antigen by splenic parenchymal $CD4^+$ T cells in mice failing immunotherapy with DISC-mGM-CSF. (Submitted).

4.Algarra I, Collado A, Garrido F. (1997). Altered MHC class I antigens in tumors. Int J Clin Lab Res.27(2):95-102.

5.Ali SA, McLean CS, Boursnell ME, Martin G, Holmes CL, Reeder S, Entwisle C, Blakeley DM, Shields JG, Todryk S, Vile R, Robins RA, Rees RC. (2000).Preclinical evaluation of "whole" cell vaccines for prophylaxis and therapy using a disabled infectious single cycle-herpes simplex virus vector to transduce cytokine genes. Cancer Res. 60(6): 1663-70.

6.Ali SA, Lynam J, McLean CS, Entwisle C, Loudon P, Rojas JM, McArdle SE, Li G, Mian S, Rees RC.(2002). Tumor regression induced by intratumor therapy with a disabled infectious single cycle (DISC) herpes simplex virus (HSV) vector, DISC/HSV/murine granulocyte-macrophage colony-stimulating factor, correlates with antigen-specific adaptive immunity. J Immunol.168(7):3512-9.

7.Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, Carbone DP, Gabrilovich DI.(2001). Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol. 166(1):678-89.

8.Antony PA, Restifo NP. (2002). Do CD4+ CD25+ immunoregulatory T cells hinder tumor immunotherapy?. J Immunother. 25(3):202-6.

9.Bai XF, Liu J, Li O, Zheng P, Liu Y. (2003). Antigenic drift as a mechanism for tumor evasion of destruction by cytolytic T lymphocytes. J Clin Invest. 111(10):1487-96.

10.Bellone G, Turletti A, Artusio E, Mareschi K, Carbone A, Tibaudi D, Robecchi A, Emanuelli G, Rodeck U. (1999). Tumor-associated transforming growth factor-beta and interleukin-10 contribute to a systemic Th2 immune phenotype in pancreatic carcinoma patients. Am J Pathol. 155(2):537-47.

11.Boursnell MEG, Entwistle C, Ali SA, Sivasubramaniam SD, Reeder SP, McLean CS, Blakeley DM, Miller J, Hill S, Shields, JG, Inglis SC, Rees RC. (1998). Disabled Infectious Single Cycle (DISC) Herpes Simplex Virus as a vector for immunotherapy of cancer. Adv. Exp. Med.Biol. 451: 379-384.
12.Bronte V, Apolloni E, Cabrelle A, Ronca R, Serafini P, Zamboni P, Restifo NP, Zanovello P. 2000. Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. Blood.*96(12):*3838-46.

13.Browning MJ, Krausa P, Rowan A, Hill AB, Bicknell DC, Bodmer JG, Bodmer WF. (1993).Loss of human leukocyte antigen expression on colorectal tumor cell lines: implications for anti-tumor immunity and immunotherapy. J Immunother.14(3):163-8 .

14.Buggins AG, Milojkovic D, Arno MJ, Lea NC, Mufti GJ, Thomas NS, Hirst WJ. (2001). Microenvironment produced by acute myeloid leukemia cells prevents T cell activation and proliferation by inhibition of NF-kappaB, c-Myc, and pRb pathways. J Immunol. 167(10):6021-30.

15.Cabrera CM, Jimenez P, Cabrera T, Esparza C, Ruiz-Cabello F, Garrido F. 2003. Total loss of MHC Class I in colorectal tumors can be explained by two molecular pathways: beta 2-microglobulin inactivation and MSI-positive tumors and LMP7/TAP2 downregulation in MS negative tumors. Tissue Antigens. Mar 61(3): 211 – 219.

16.Cabrera T, Lopez-Nevot MA, Gaforio JJ, Ruiz-Cabello F, Garrido F. (2003). Analysis of HLA expression in human tumor tissues. Cancer Immunol Immunother. 52: 1-9.

17.Cabrera T, Maleno I, Lopez-Nevot MA, Redondo M, Fernandez MA, Collado A, Garrido F. 2003. High frequency of HLA- B44 allelic loss in human solid tumors. Hum Immunol. Oct 64(10): 941-50.

18.Cabrera T, Pedrajas G, Cozar JM, Garrido A, Vincente J, Tallada M, Garrido F. 2003. HLA class I expression in bladder carcinomas. Tissue Antigens. Oct 62 (4): 324 – 7.

19.Chen L, McGowan P, Ashe S, Johnston J, Li Y, Hellstrom I, Hellstrom KE. (1994). Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. J Exp Med.179(2):523-32.

20.Chen YL, Chen SH, Wang JY, Yang BC. (2003). Fas ligand on tumor cells mediates inactivation of neutrophils. J Immunol. 2003 Aug 1;171(3):1183-91.

21.Ciavarra RP, Brown RR, Holterman DA, Garrett M, Glass WF 2nd, Wright GL Jr, Schellhammer PF, Somers KD.Impact of the tumor microenvironment on host infiltrating cells and the efficacy of flt3-ligand combination immunotherapy evaluated in a treatment model of mouse prostate cancer. Cancer Immunol Immunother. 2003 Jun 24.

22.Ciurea A, Hunziker L, Martinic MM, Oxenius A, Hengartner H, Zinkernagel RM. (2001).CD4+ T-cell-epitope escape mutant virus selected in vivo. Nat Med. 7:795-800.

23.Clark RE, Dodi IA, Hill SC, Lill JR, Aubert G, Macintyre AR, Rojas J, Bourdon A, Bonner PL, Wang L, Christmas SE, Travers PJ, Creaser CS, Rees RC, Madrigal JA. (2001). Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. Blood.98(10):2887-93.

24.Davidson WF, Giese T, Fredrickson TN. (1998). Spontaneous development of plasmacytoid tumors in mice with defective Fas-Fas ligand interactions. Exp Med. 187(11):1825-38.

25.De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M. (1997). Effect of interleukin-10 on dendritic cell maturation and function. Eur J Immunol. 27(5):1229-35.

26.de Vries TJ, Fourkour A, Wobbes T, Verkroost G, Ruiter DJ, van Muijen GN. (1997). Heterogeneous expression of immunotherapy candidate proteins gp100, MART-1, and tyrosinase in human melanoma cell lines and in human melanocytic lesions. Cancer Res. 57(15):3223-9.

27.Doran T, Stuhlmiller H, Kim JA, Martin EW Jr, Triozzi PL. (1997). Oncogene and cytokine expression of human colorectal tumors responding to immunotherapy. J Immunother. 20(5):372-6.

28.Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol.3(11):991-8.

29.Dye ES, North RJ. (1981). T cell-mediated immunosuppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. J Exp Med.154(4):1033-42.

30.Fan XH, Han BH, Dong QG, Sha HF, Bao GL, Liao ML.(2003). Vascular endothelial growth factor inhibits dendritic cells from patients with NSCLC. Zhonghua Jie He He Xi Za Zhi. (9): 539 – 43.

31.Feenstra M, Verdaasdonk M, van der Zwan AW, de Weger R, Slootweg P, Tilanus M. (2000). Microsatellite analysis of microdissected tumor cells and 6p high density microsatellite analysis in head and neck squamous cell carcinomas with down-regulated human leukocyte antigen class I expression. Lab Invest.80(3):405-14.

32.Fennell DA. (2003).Bcl-2 as a target for overcoming chemoresistance in small-cell lung cancer. Clin Lung Cancer.4(5):307-13.

33.Fortis C, Foppoli M, Gianotti L, Galli L, Citterio G, Consogno G, Gentilini O, Braga M. (1996). Increased interleukin-10 serum levels in patients with solid tumors. Cancer Lett. 104(1):1-5.

34.Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. (2002). Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med.196(4):459-68.

35.Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM. (2001). Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. J Immunol.166(9):5398-406.

36.Gaken J, Jiang J, Daniel K, van Berkel E, Hughes C, Kuiper M, Darling D, Tavassoli M, Galea-Lauri J, Ford K, Kemeny M, Russell S, Farzaneh F. (2000). Fusagene vectors: a novel strategy for the expression of multiple genes from a single cistron. Gene Ther. 7(23): 1979-85.

37.Garrido F, Algarra I. (2001). MHC antigens and tumor escape from immune surveillance. Adv. Cancer Res. 83:117-158.

38.Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA. (1992). Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. Cancer Res. 52(24):6949-52.

39.Griffioen M, Ouwerkerk IJ, Harten V, Schrier PI. (2000). HLA-B locus-specific downregulation in human melanoma requires enhancer A as well as a sequence element located downstream of the transcription initiation site. Immunogenetics.52(1-2):121-8.

40.Groneberg C, Pickartz T, Binder A, Ringel F, Srock S, Sieber T, Schoeler D, Schriever F. (2003). Clinical relevance of CD95 (Fas/Apo-1) on T cells of patients with B-cell chronic lymphocytic leukemia. Exp Hematol.31(8):682-5.

41.Gruss HJ, Dower SK.(1995). Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. Blood. 85: 3378-404.

42.Hersey P, Zhang XD. (2001). How melanoma cells evade trail-induced apoptosis. Nat Rev Cancer. 1(2):142-50.

43.Hicklin DJ, Francesco M, Marincola FM, Ferrone S.(1999).HLA Class I antigen downregulation in human cancers: T – cell immunotherapy revives an old story. Molecular Medicine Today 5: 178 – 186.

44.Hofbauer GF, Kamarashev J, Geertsen R, Boni R, Dummer R. (1998). Melan A/MART-1 immunoreactivity in formalin-fixed paraffin-embedded primary and metastatic melanoma: frequency and distribution. Melanoma Res.8(4):337-43.

45.Ikeda H, Lethe B, Lehmann F, van Baren N, Baurain JF, de Smet C, Chambost H, Vitale M, Moretta A, Boon T, Coulie PG. (1997). Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity.6(2):199-208.

46.Inoshima N, Nakanishi Y, Minami T, Izumi M, Takayama K, Yoshin Hara N. (2002). The influence of dendritic cell infilteration and VEGF expression on the prognosis of NSCLC. Clin Cancer Res. 8(11): 3480-6.

47.Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J. (1997). Inhibition of death receptor signals by cellular FLIP. Nature. 388(6638): 190-5.

48.Jager E, Jager D, Knuth A.(2000). CTL-defined cancer vaccines in melanoma and other epithelial cancer. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp 207.

49. Jakobisiak M, Lasek W, Golab J. (2003). Natural mechanisms protecting against cancer. Immunol Lett. 90(2-3): 103-22.

50.Janeway CA, Travers P, Walport M, Shlomchik M. (2002). Immunobiology of disease. 5th Edition. Garland Publishing, USA.

51.Javia LR, Rosenberg SA.(2003). CD4+CD25+ suppressor lymphocytes in the circulation of patients immunized against melanoma antigens. J Immunother. 26:85-93.

52.Johnsen AK, France J, Nagy N, Askew D, Abdul-Karim FW, Gerson SL, Sy MS, Harding CV. (2001). Systemic deficits in transporter for antigen presentation (TAP)-1 or proteasome subunit LMP2 have little or no effect on tumor incidence. Int J Cancer. 91(3):366-72.

53.Kase H, Aoki Y, Tanaka K. (2003). Fas ligand expression in cervical adenocarcinoma: relevance to lymph node metastasis and tumor progression. Gynecol Oncol. 90(1):70-4.

54.Kaufmann HL, Schlorn J. (2000). Vaccines in colon cancer. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp 107.

55.Keating PJ, Cromme FV, Duggan-Keen M, Snijders PJ, Walboomers JM, Hunter RD, Dyer PA, Stern PL. (1995). Frequency of downregulation of individual HLA-A and –B alleles in cervical carcinomas in relation to TAP-1 expression. Br.J.Cancer. 72 (2):405-411.

56.Khong HT, Restifo NP.(2002). Natural selection of tumor variants in the generation of "tumor escape" phenotypes. Nat Immunol.3(11):999-1005.

57.Kono K, Salazar-Onfray F, Petersson M, Hansson J, Masucci G, Wasserman K, Nakazawa T, Anderson P, Kiessling R. (1996). Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell-and natural killer cell-mediated cytotoxicity. Eur J Immunol. 26(6):1308-13.

58.Koopman LA, Corver WE, van der Slik AR, Giphart MJ, Fleuren GJ (2000). Multiple genetic alterations cause frequent and heterogeneous human histocompatibility leukocyte antigen class I loss in cervical cancer. J. Exp. Med. 191:961-975.

59.Kovacsovics-Bankowski M, Rock KL. (1995). A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. Science 267(5195): 243-6.

60.Krasagakis K, Tholke D, Farthmann B, Eberle J, Mansmann U, Orfanos CE. (1998). Elevated plasma levels of transforming growth factor (TGF)-beta1 and TGF-beta2 in patients with disseminated malignant melanoma. Br J Cancer. 77(9):1492-4.

61.Kusmartsev SA, Li Y, Chen SH. (2000). Gr-1+ myeloid cells derived from tumor-bearing mice inhibit primary T cell activation induced through CD3/CD28 costimulation. J Immunol.165(2):779-85.

62. Le YS, Kim TE, Kim BK, Park YG, Kim GM, Jee SB, Ryu KS, Kim IK, Kim JW. (2002). Alterations of HLA Class I and Class II antigen expression in borderline invasive and metastatic ovarian cancers. Exp Mol Med . 34(1): 18-26.
63.Levey DL, Srivastava PK.(1996). Alterations in T cells of cancer-bearers: whence specificity? Immunol Today.17:365-8.

64.Li R, Ruttinger D, Li R, Si LS, Wang YL.(2003).Analysis of the immunological microenvironment at the tumor site in patients with non-small cell lung cancer. Langenbecks Arch Surg. Sep 19 [epub ahead of print].

65.Lissoni P, Malugani F, Bonfanti A, Bucovec R, Secondino S, Brivio F, Ferrari – Bravo A, Ferrante R, Vigore L, Rovelli F, Mandala M, Vivian Fumagalli L, Gardani GS. (2001). Abnormally enhanced blood concentrations of VEGF in metastatic cancer patients and their relation to circulating dendritic cells, IL-12 and endothelin-1. J Biol Regul Homeost Agents. (2): 140-4.

66.Lozupone F, Rivoltini L, Luciani F, Venditti M, Lugini L, Cova A, Squarcina P, Parmiani G, Belardelli F, Fais S. (2003). Adoptive transfer of an anti-MART-1(27-35)-specific CD8+ T cell clone leads to immunoselection of human melanoma antigen-loss variants in SCID mice. Eur J Immunol. 33(2):556-66.

67.Maeda H, Shiraishi A. (1996). TGF-beta contributes to the shift toward Th2-type responses through direct and IL-10-mediated pathways in tumor-bearing mice. J Immunol. 156(1):73-8.

68.Maeurer MJ, Gollin SM, Martin D, Swaney W, Bryant J, Castelli C, Robbins P, Parmiani G, Storkus WJ, Lotze MT.(1996). Tumor escape from immune recognition: lethal recurrent melanoma in a patient associated with downregulation of the peptide transporter protein TAP-1 and loss of expression of the immunodominant MART-1/Melan-A antigen. J Clin Invest. 98(7):1633-41.

69. Maiorana A, Cesinaro AM, Fano RA, Collina G. (1995). Expression of MHC Class I and Class II antigens in primary breast carcinomas and synchronous nodal metastases. Clin Exp Metastasis. 13(1): 43-8.

70.Matzinger P. Tolerance, danger, and the extended family. (1994). Annu Rev Immunol. 12: 991-1045

71.Mazzoni A, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P, Zanovello P, Segal DM. (2002). Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. J Immunol.168(2):689-95.

72.McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. (2002). CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity. 2002 Feb;16(2):311-23.

73.Medema JP, de Jong J, van Hall T, Melief CJ, Offringa R. (1999). Immune escape of tumors in vivo by expression of cellular FLICE-inhibitory protein. J Exp Med.190(7):1033-8.

74.Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. (1998). Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat Med.(5): 581-7.

75.Melero I, Shuford WW, Newby SA, Aruffo A, Ledbetter JA, Hellstrom KE, Mittler RS, Chen L. (1997). Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med. 3(6):682-5.

76.Mellor AL, Munn DH. (1999). Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? Immunol Today.20(10):469-73.

77.Mukherjee P, Ginardi AR, Madsen CS, Tinder TL, Jacobs F, Parker J, Agrawal B, Longenecker BM, Gendler SJ. (2001). MUC1-specific CTLs are non-functional within a pancreatic tumor microenvironment. Glycoconj J. 18(11-12):931-42.

78.Muller L, Kiessling R, Rees RC, Pawelec G. (2002). Muller L, Kiessling R, Rees RC, Pawelec G. J Environ Pathol Toxicol Oncol.21(4):277-330.

79.Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL Jr, Mellor AL. (2002).Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science.297(5588):1867-70.

80.Nakagomi H, Petersson M, Magnusson I, Juhlin C, Matsuda M, Mellstedt H, Taupin JL, Vivier E, Anderson P, Kiessling R. (1993). Decreased expression of the signal-transducing zeta chains in tumor-infiltrating T-cells and NK cells of patients with colorectal carcinoma. Cancer Res. 53(23):5610-2.

81.Nieland JD, Loviscek K, Kono K, Albain KS, McCall AR, Potkul RK, Fisher SG, Velders MP, Petersson M, Kiessling R, Kast WM. (1998). PBLs of early breast

carcinoma patients with a high nuclear grade tumor unlike PBLs of cervical carcinoma patients do not show a decreased TCR zeta expression but are functionally impaired. J Immunother. 21(4):317-322.

82.Ohm JE, Carbone DP. (2001).VEGF as a mediator of tumor associated immunodeficiency. Immunol Res. 23 (2-3): 263-72.

83.Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T. (1996). Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 zeta chain of T-cell receptor complex and antigen-specific T-cell responses. Proc Natl Acad Sci U S.A. 93(23):13119-24.

84.Osaki M, Kase S, Adachi K, Takeda A, Hashimoto K, Ito H. (2003).Inhibition of the PI3K-Akt signaling pathway enhances the sensitivity of Fas-mediated apoptosis in human gastric carcinoma cell line, MKN-45. J Cancer Res Clin Oncol. Nov 7 [Epub ahead of print].

85.Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP, Gabrilovich DI. (1998). VEGF affects DC maturation through the inhibition of NF-kB activation in haemopoietic cells. J Immunol. 160(3): 1224-32.

86.Pardoll DM. (1998). Cancer Vaccines. Nature Medicine Suppl. 4:525-531.

87.Pardoll, D. (2001). T cells and tumors. Nature. 411:1010-1012 (12).

88.Perez B, Benitez R, Fernandez MA, Oliva MR, Soto JL, Serrano S, Lopez Nevot MA, Garrido F. (1999). A new beta 2 microglobulin mutation found in a melanoma tumor cell line. Tissue Antigens.53(6):569-72.

89.Plescia OJ, Grinwich K, Plescia AM.(1976). Subversive activity of syngeneic tumor cells as an escape mechanism from immune surveillance and the role of prostaglandins. Ann N Y Acad Sci. 276:455-65.

90.Ragnarsson GB, Mikaelsdottir EK, Vidarsson H, Jonasson JG, Olafsdottir K, Kristjansdottir K, Kjartansson J, Ogmundsdottir HM, Rafnar T. (2000). Intracellular Fas ligand in normal and malignant breast epithelium does not induce apoptosis in Fas-sensitive cells. Br J Cancer.;83(12):1715-21.

91.Ramal LM, Feenstra M, van der Zwan AW, Collado A, Lopez-Nevot MA, Tilanus M, Garrido F. (2000). Criteria to define HLA haplotype loss in human solid tumors. Tissue Antigens.55(5):443-8.

92.Ramal LM, Maleno I, Cabrera T, Collado A, Ferron A, Lopez-Nevot MA, Garrido F. (2000). Molecular strategies to define HLA haplotype loss in microdissected tumor cells. Hum Immunol. 2000 Oct;61(10):1001-12.

93.Real LM, Jimenez P, Kirkin A, Serrano A, Garcia A, Canton J, Zeuthen J, Garrido F, Ruiz-Cabello F. (2001).Multiple mechanisms of immune evasion can coexist in melanoma tumor cell lines derived from the same patient. Cancer Immunol Immunother. Jan;49(11):621-8.

94.Rees RC, McArdle S, Mian S, Li G, Ahmad M, Parkinson R, Ali SA. (2002). Disabled Infectious Single Cycle- Herpes Simplex Virus (DISC-HSV) as a vector for immunogene therapy of cancer. Curr Opin Mol Ther. 4:49-53.

95.Restifo NP, Marincola FM, Kawakami Y, Taubenberger J, Yannelli JR, Rosenberg SA. (1996). Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. J Natl Cancer Inst. 88(2):100-8.

96.Restifo NP.(1996). The new vaccines: building viruses that elicit antitumor immunity. Curr Opin Immunol. 8(5):658-63.

97.Rivoltini L, Carrabba M, Huber V, Castelli C, Novellino L, Dalerba P, Mortarini R, Arancia G, Anichini A, Fais S, Parmiani G. (2002). Immunity to cancer: attack and escape in T lymphocyte-tumor cell interaction. Immunol Rev.188:97-113.

98.Rohrlich PS, Cardinaud S, Firat H, Lamari M, Briand P, Escriou N, Lemonnier FA.(2003).HLA-B*0702 transgenic, H-2KbDb double-knockout mice: phenotypical and functional characterization in response to influenza virus. Int Immunol.15:765-72.

99.Romero P. (2003). IL-31 Monitoring natural and vaccine induced T cell responses in melanoma. Pigment Cell Res.16(5):588.

100.Rudolf MP, Small LA, Velders MP, DaSilva DM, Weijzens S, Kast MW. (2000). Vaccine delivery and immunosuppression in cervical cancer. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp82.

101.Salvesen GS, Dixit VM. (1999). Caspase activation: the induced-proximity model. Proc Natl Acad Sci U S A. 96(20):10964-7.
102.Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A. (2003). CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. Cancer. 98:1089-99.

103.Schule J, Bergkvist L, Hakansson L, Gustafsson B, Hakansson A. (2002). Down-regulation of the CD3-zeta chain in sentinel node biopsies from breast cancer patients. Breast Cancer Res Treat. 74(1):33-40.

104.Schwartz RH. (1990). A cell culture model for T lymphocyte clonal anergy. Science. 248(4961):1349-56.

105.Serrano A, Tanzarella S, Lionello I, Mendez R, Traversari C, Ruiz-Cabello F, Garrido F. (2001). Rexpression of HLA class I antigens and restoration of antigen-specific CTL response in melanoma cells following 5-aza-2'-deoxycytidine treatment. Int J Cancer.94(2):243-51.

106.Sharma S, Stolina M, Lin Y, Gardner B, Miller PW, Kronenberg M, Dubinett SM. (1999). T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. J Immunol. 163(9):5020-28.

107. Sharpe JC, Abel PD, Gilbertson JA, Brawn P, Foster CS. (1994). Modulated expression of human leucocyte antigen class I and class II determinants in hyperplastic and malignant human prostatic epithelium. Br J Urol. 74(5): 609-16.

108.Smith CA, Farrah T, Goodwin RG. (1994). The TNF receptor superfamily of cellular and viral proteins: Activation, costimulation, and death. Cell. 76: 959 - 962.

109.Song E, Chen J, Ouyang N, Su F, Wang M, Heemann U. (2001). Soluble Fas ligand released by colon adenocarcinoma cells induces host lymphocyte apoptosis: an active mode of immune evasion in colon cancer. Br J Cancer. 85(7):1047-54.

110.Soong TW, Hui KM. (1991). Regulation of the expression of major histocompatibility complex class I genes in human colorectal cancer. Cancer Detect Prev.15(3):231-9.

111.Speiser DE, Rimoldi D, Batard P, Lienard D, Lejeune F, Cerottini JC, Romero P. (2003). Disease-driven T cell activation predicts immune responses to vaccination against melanoma. Cancer Immunol.3:12.

112.Stevenson FK, Zhu D, Spellerberg, M, King CA, Sahota SS, Rice J, Thompsett AR, Hamblin TJ. (2000). DNA vaccines against B-cell tumors. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp 218.

113.Sutmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, Toes RE, Offringa R, Melief CJ. (2001). Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J Exp Med. 194(6):823-32.

114.Swanson KA, Zheng Y, Heidler KM, Mizobuchi T, Wilkes DS. (2003). CD11c+ Cells Modulate Pulmonary Immune Responses by Production of Indoleamine 2,3-dioxygenase1. Am J Respir Cell Mol Biol. Sep 4 [Epub ahead of print].

115.Takeda K, Smyth MJ, Cretney E, Hayakawa Y, Kayagaki N, Yagita H, Okumura K. (2002). Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. J Exp Med.195(2):161-9.

116.Takeda K, Smyth MJ, Cretney E, Hayakawa Y, Yamaguchi N, Yagita H, Okumura K. (2001). Involvement of tumor necrosis factor-related apoptosis-inducing ligand in NK cell-mediated and IFN-gamma-dependent suppression of subcutaneous tumor growth. Cell Immunol. 214(2):194-200.

117.Taylor MW, Feng GS. (1991).Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J. 5(11):2516-22.

118.Taylor Papadimitriou J, Burchell J, Miles DW. (2000). MUC1 vaccines and breast cancer. In: Cancer Vaccines & Immunotherapy. Eds. Stern PL, Beverely PCL, Carroll MW. Cambridge University Press pp 135.

119.Thornton AM, Shevach EM.(2000). Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol. 164: 183-90.

120.Todryk S, Ali SA, Dalgleish A, Rees RC. (2001). Genetically modified tumor cells for cancer immunisation. In: Cancer Immunology. Eds. Robins RA and Rees RC. Kluwer Academic Publishers. Netherlands pp 181-194.

121.Toi M, Taniguchi T, Yamamoto Y, Kurisaki T, Suzuki H, Tominaga T. (1996). Clinical significance of the determination of angiogenic factors. Eur J Cancer. 32A(14):2513-9.

122.Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, Imai Y, Shimomukai H, Nomura Y, Matsuda Y, Matsuzawa Y. (1996). High levels of transforming growth factor beta 1 in patients with colorectal cancer: association with disease progression. Gastroenterology. 110(2): 375-82.

123. Ugurel S, Uhlig D, Pfohler C, Tilgen W, Schadendorf D, Reinhold U. (2004). Down-regulation of HLA Class II and co-stimulatory CD86/B7.2 on circulating monocytes from melanoma patients. Cancer Immunol Immunother. Jan 16 (Epub ahead of print).

124.Uyttenhove C, Godfraind C, Lethe B, Amar-Costesec A, Renauld JC, Gajewski TF, Duffour MT, Warnier G, Boon T, Van den Eynde BJ. (1997). The expression of mouse gene P1A in testis does not prevent safe induction of cytolytic T cells against a P1A-encoded tumor antigen. Int J Cancer. 70(3):349-56.

125.Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, Boon T, Van Den Eynde BJ.(2003).Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med.9(10):1269-74.

126.von Bernstorff W, Voss M, Freichel S, Schmid A, Vogel I, Johnk C, Henne-Bruns D, Kremer B, Kalthoff H. (2001). Systemic and local immunosuppression in pancreatic cancer patients. Clin Cancer Res.7(3 Suppl):925s-932s.

127.Weinberg AD, Rivera MM, Prell R, Morris A, Ramstad T, Vetto JT, Urba WJ, Alvord G, Bunce C, Shields J. (2000). Engagement of the OX-40 receptor in vivo enhances antitumor immunity. J Immunol. 164(4):2160-9.

128.Wen XY, Mandelbaum S, Li ZH, Hitt M, Graham FL, Hawley RG, Stewart AK. (2001). Tricistronic viral vectors co-expressing interleukin 12 (IL-12) and CD80 (B7.1) for the immunotherapy of cancer: preclinical studies in myeloma. Cancer Gene Ther. 8(5): 361-70.

129.Wilcox RA, Flies DB, Zhu G, Johnson AJ, Tamada K, Chapoval AI, Strome SE, Pease LR, Chen L. (2002). Provision of antigen and CD137 signaling breaks immunological ignorance, promoting regression of poorly immunogenic tumors. J Clin Invest. 109(5):651-9.

130.Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, Rubin SC, Kaiser LR, June CH. (2001).Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res. 61(12):4766-7.

131.Zheng P, Sarma S, Guo Y, Liu Y.(1999). Two mechanisms for tumor evasion of preexisting cytotoxic T-cell responses: lessons from recurrent tumors. Cancer Res. 59(14):3461-7.