Mechanisms of Immune Evasion by Tumors

Charles G. Drake, Elizabeth Jaffee, and Drew M. Pardoll

Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Abstract 51
1. Introduction 51
2. Downmodulation of Tumor Antigen Presentation 52
3. Immunologic Barriers Within the Tumor Microenvironment 55
4. Disabled Antigen Presenting Cells 62
5. CD4 T-Cell Tolerance 63
6. Coinhibition 64
7. Regulatory T Cells 66
8. CD8 T-Cell Dysfunction 68
9. Summary 69
References 70

Abstract

In the past decade, basic studies in animal models have begun to elucidate the physiological barriers which impede a successful antitumor immune response. These barriers operate at a number of levels, and involve the tumor, the tumor microenvironment and various components of the innate and adaptive immune systems. In this review, we discuss the multiple mechanisms by which tumors evade an immune response, with an emphasis on clinically relevant strategies to overcome these inhibitory checkpoints.

1. Introduction

While there has been recent focus on regulatory and organizational barriers to the development of successful antitumor immunotherapy (Pardoll and Allison, 2004), we must not lose sight of the multitude of physiological barriers to generation of successful antitumor immune responses that must be overcome before cancer immunotherapy strategies can hope to be highly efficacious in humans. In the past decade, basic studies in animal models have begun to elucidate these immunologic barriers and to suggest potential compensatory interventions. These barriers operate at a number of different levels along the pathway from induction to successful execution of antitumor immune responses, and multiple checkpoints may be in place simultaneously. In a general sense, one can divide immune evasion by tumors into two general categories: (1) tolerance induction by the developing tumor and (2) resistance to killing by activated immune effector cells. Each of these general categories can be

ADVANCES IN IMMUNOLOGY, VOL. 90 0065-2776/06 $35.00
© 2006 Elsevier Inc. All rights reserved.
DOI: 10.1016/S0065-2776(06)90002-9

subdivided into multiple mechanisms, some of which are becoming elucidated at the molecular level. For example, tumor-induced tolerance could occur because the tumor is "ignored" by the immune system or because the tumor somehow actively induces anergy among tumor-specific T cells, reduces regulatory T cells or mediates deletion of tumor-specific T cells. If tumor tolerance were predominantly due to ignorance, therapeutic tumor vaccination by itself would likely be much more effective clinically than experience has demonstrated. With occasional exceptions, the majority of murine tumor studies indicate that a more active form of tumor-induced tolerance appears to be operative. While deletion of tumor reactive T cells may indeed be occurring, there is ample evidence that significant repertoires of tumor-specific T cells remain, albeit in an anergic or suppressed state. A critical issue relevant to prospects for immunotherapy is the reversibility of this anergic/inactive state achievable through specific interventions.

This review provides an overview of the recently described checkpoints to antitumor immunity that represent biologic barriers to successful immunotherapy, with an emphasis on those that operate at the T cell level.

2. Downmodulation of Tumor Antigen Presentation

Immune recognition of tumors as distinct from their normal tissue counterparts fundamentally depends on T cell recognition of tumor-specific or tumor-associated antigens. It is now well established that tumors differ from their normal cell counterparts in antigenic composition. The molecular hallmark of carcinogenesis is genetic instability (Fearon and Vogelstein, 1990). Genetic instability in cancers is a consequence of deletion and/or mutational inactivation of genome guardians such as p53 (Lu and Lane, 1993). New antigens are constantly being generated in tumors as a consequence of genetic instability as the carcinogenic process develops and progresses. This does not occur in normal, nontransformed tissues, which maintain a very stable antigenic profile. In addition to the thousands of mutational events that occur during tumorigenesis, epigenetic changes as a consequence of hyper- and hypo-methylation alter expression levels of hundreds of genes (Jones and Baylin, 2002). While these epigenetic changes do not formally create tumor-specific neoantigens, they raise the concentration of encoded proteins many orders of magnitude, thereby dramatically affecting antigenicity.

The first mechanism of tumor immune evasion to be recognized and studied involved the inhibition of tumor antigen presentation (Fig. 1). Downregulation of the antigen processing machinery—particularly the MHC class I pathway—has been documented extensively in a large variety of tumors. In the human, downregulation of the MHC class I molecules has been observed in a diversity

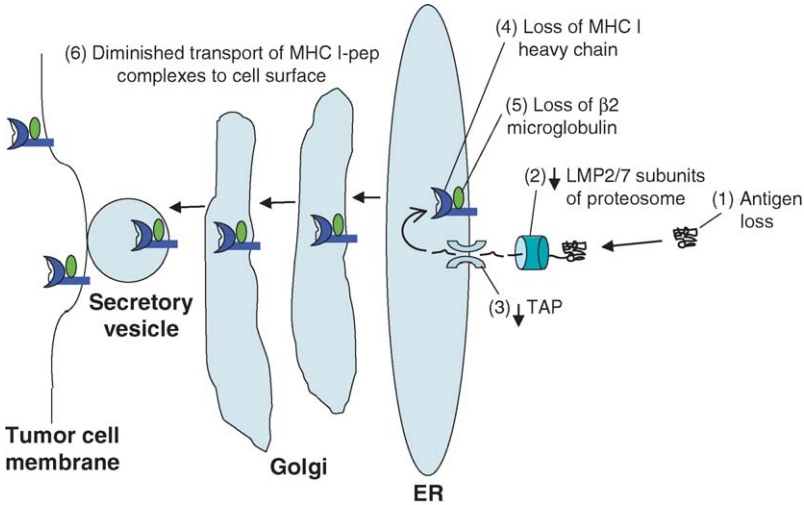

Figure 1 Tumors can downmodulate multiple components of the MHC I processing pathway in order to avoid recognition by tumor specific CTL. In addition to antigen loss, downmodulation of proteosome subunits, transporter associated with antigen presentation (TAP), β2 microglobulin, and MHC I heavy chain can diminish presentation of MHC-peptide complexes on the tumor surface as a means of evasion of CTL recognition. In addition, transport of MHC-peptide complexes from endoplasmic reticulum (ER) through the Golgi to the cell membrane can be diminished. While mutation or deletion has been documented in some tumors, most examples of downregulation of MHC I processing and presentation components are epigenetic and reversible with interferon treatment.

of tumor types, such as breast cancer, prostate cancer, and lung cancer (Cabrera *et al.*, 1996, 1998; Esteban *et al.*, 1989, 1990, 1996; Ferrone and Marincola, 1995; Marincola *et al.*, 2000; Natali *et al.*, 1983, 1984, 1985, 1989; Perez *et al.*, 1986; Ruiz-Cabello *et al.*, 1989, 1991; van den Ingh *et al.*, 1987; van Driel *et al.*, 1996; Whitwell *et al.*, 1984; Zuk, 1987). In many cases, individual HLA alleles are selectively lost and this has been suggested to represent downmodulation of presentation of immunodominant tumor antigens; however, this notion has never been directly proven. Global MHC class I loss or downmodulation has also been observed in tumors. The most common mechanism for global MHC class I loss is mutations + deletion of β2-microglobulin genes (Benitez *et al.*, 1998; D'Urso *et al.*, 1991; Wang *et al.*, 1993). Loss of heterozygosity at the β2-microglobulin locus with mutation of the remaining allele is the typical scenario. Downmodulation of MHC class I genes can result from multiple mechanisms affecting transcription (Blanchet *et al.*, 1992; Doyle *et al.*, 1985). Downregulation of TAP genes as well as components of the immunoproteosome, such as LMP-2 and LMP-7, have

likewise been documented in a number of tumor types (Alpan *et al.*, 1996; Hilders *et al.*, 1994; Restifo *et al.*, 1996; Rowe *et al.*, 1995; Sanda *et al.*, 1995; Seliger *et al.*, 1996). In the majority of cases where the MHC class I processing machinery is downmodulated, it is usually rapidly upregulated by γ-IFN, suggesting that the diminished expression is epigenetic in origin and reversible. Not infrequently, tumors express higher levels of MHC class I molecules and processing machinery than their normal tissue of origin. For example, virtually all renal cancers express quite high levels of MHC class I, whereas normal renal epithelium expresses barely detectable levels of surface MHC class I and very low levels of TAP until exposed to stimuli such as γ-IFN.

Attempts to correlate levels of MHC expression with clinical prognosis in humans or tumor growth rates in the mouse have generated inconsistent outcomes, depending on the tumor type or system. Some human studies suggest that expression of MHC molecules by the tumor is a poor prognostic indicator. Other studies have suggested that high expression of HLA molecules correlates with a favorable prognosis. An example of human cancer in which MHC class I level is consistently downmodulated by multiple mechanisms in the progression from premalignant lesions to malignancy is cervical cancer (Clarke and Chetty, 2002; Koopman *et al.*, 2000). This may be due to the viral (HPV) etiology and the fact that most premalignant HPV lesions are naturally eliminated in immunocompetent but not immunocompromised individuals (Moretta *et al.*, 1997).

Needless to say, since NK cells demonstrate enhanced recognition and killing of cells with low MHC class I levels (Hui *et al.*, 1984; Lanier and Phillips, 1996), downmodulation of the MHC class I processing machinery would not necessarily represent an effective strategy by the tumor to cloak itself from recognition by the immune system. Indeed, while some reports suggested that increasing the level of MHC expression resulted in diminished *in vivo* tumor growth of some murine tumors (Haywood and McKhann, 1971; Ljunggren and Karre, 1985; Wallich *et al.*, 1985), other tumors demonstrate exactly the opposite outcome—namely, diminished growth with lower levels of MHC expression consequent to enhanced NK cell recognition (Karre *et al.*, 1986; Urban *et al.*, 1982). In conclusion, while the modulation of MHC levels and antigen processing machinery is often observed during the progression of cancer, it is yet unclear whether this is a true consequence of development of immune resistance in response to a robust immune surveillance system.

Arguments about loss of tumor-associated antigens (TAA) as an escape mechanism from immune surveillance are equally inconclusive. Heterogeneity of TAA expression and attempts to correlate TAA loss are well documented in murine tumor models with transplantation of immunogenic tumors or after vaccination (Lethe *et al.*, 1992; Urban *et al.*, 1986; Uyttenhove *et al.*, 1983; Ward

et al., 1989; Wortzel *et al.*, 1983; Yee *et al.*, 2002). Likewise, Yee *et al.* demonstrated specific loss of cognate melanoma antigens in relapsing tumors from patients treated with adoptive transfer of melanoma antigen-specific $CD8^+$ T cells (Jager *et al.*, 1997). Similarly, there are anecdotal reports of specific antigen loss after treatment of melanoma patients with peptide vaccines (de Vries *et al.*, 1998; Ohnmacht *et al.*, 2001). Together, these reports support the concept of TAA loss as a robust mechanism to escape immunotherapeutically induced antitumor responses. However, despite attempts to document TAA loss with natural tumor progression in humans (particularly in melanoma) (Cormier *et al.*, 1998, 1999; Riker *et al.*, 1999; Scheibenbogen *et al.*, 1996; Schmid *et al.*, 1995), there is no clear evidence that TAA loss is a tumor escape response to immune surveillance in the unmanipulated host.

3. Immunologic Barriers Within the Tumor Microenvironment

While uncontrolled growth is certainly a common biological feature of all tumors, the major pathophysiologic characteristics of malignant cancer responsible for morbidity and mortality are the ability to invade across natural tissue barriers and to metastasize. Both of these characteristics, which are never seen with normal tissues or benign tumors, are associated with dramatic disruption of tissue architecture. One of the important consequences of tissue disruption, even when caused by noninfectious mechanisms, is the elaboration of proinflammatory signals. These signals, generally in the form of cytokines and chemokines, are central initiators of both innate and adaptive immune responses. Thus, unlike normal tissues, cancers are constantly confronted with potential inflammatory responses as they invade tissues and metastasize through the body. How they handle and modulate these responses dictates the interplay with the host immune system. Despite the potential inflammatory and immunogenic consequences of tissue disruption associated with tissue invasion by tumors, the immune system is generally tolerant to established cancers.

In fact, chronic inflammatory responses in the tumor microenvironment not only typically fail to result in tumor elimination, but rather, can also enhance the transformation and growth of tumors. NFκB signaling in hematopoietic cells has been reported to play a critical procarcinogenic role. Selective IKK-β knockout in macrophages and neutrophils resulted in decreases in both number and growth rate of carcinogen-induced tumors in a carcinogen-induced colon cancer model. The general hypothesis to explain these results is that NFκB activation within infiltrating hematopoietic elements is thought to result in production of various proinflammatory cytokines and chemokines, a number of which enhance proliferative responses among IECs as well as providing an antiapoptotic effect (Greten *et al.*, 2004; Karin and Greten, 2005). In

particular, these include cytokines, such as TNF, IL-1, IL-6, and CSF-1, type I interferons and multiple chemokines (e.g., IL-8). These proinflammatory cytokines/chemokines have been proposed to promote carcinogenesis in a number of ways, though specific mechanistic details remain to be worked out. Cytokines, such as TNF, can induce an NFκB cascade in adjacent epithelial cells (Lind *et al.*, 2004). IL-6 and CSF-1 can act as growth factors in some cases (Klein *et al.*, 1995; Lin *et al.*, 2002). In addition, NFκB-dependent activation of certain hematopoietic elements, such as macrophages, will induce production of reactive nitrogen species (i.e., NO) through induction of iNOS. Activation of the cytochrome oxidase and NADPH oxidase pathway in neutrophils can result in production of reactive oxygen species (ROS). In some cases, the combination of reactive oxygen and nitrogen species can result in the production of peroxynitrites. These are all highly chemically reactive molecules that can cause oxidative stress in adjacent tumor cells (Bakkenist and Kastan, 2004). The result of ROS and RNS production can be tumoricidal in certain cases but can also promote carcinogenesis through induction of DNA damage under circumstances where the DNA damage response has been disabled, as is commonly the case in cancers.

Together, the accumulating body of information demonstrates that inflammation within the tumor microenvironment, as defined by infiltrating hematopoietically derived cells, can either be bad for the tumor or promote its development and growth. In striking contrast to the inflammatory response associated with infection, the inflammatory response within a tumor does not appear to result in activation of adaptive antitumor immunity. As described later, experiments employing TCR transgenic mice have provided strong evidence for the capacity of tumor cells to induce tolerance to their antigens. Tolerance appears to be operative predominately at the level of T cells; B-cell tolerance to tumors is less certain since there is ample evidence for the induction of antibody responses in animals bearing tumors as well as human patients with tumors. Numerous adoptive transfer studies have demonstrated the potent capacity of T cells to kill growing tumors, either directly through CTL activity or indirectly through multiple CD4-dependent effector mechanisms. It is thus likely that from the tumor's standpoint, induction of antigen-specific tolerance among T cells is of paramount importance for survival.

Mounting evidence supports the idea that tumors actively inhibit the release and sensing of danger signals in order to invade tissues and metastasize without evoking antitumor immune responses that would inhibit their growth, thereby converting inflammatory responses to those that could instead potentiate tumor growth (Fig. 2). Studies have in fact demonstrated that oncogenic signaling pathways not only promote tumor growth and survival in a cell-intrinsic fashion but also actively modulate their immunologic microenvironment. The

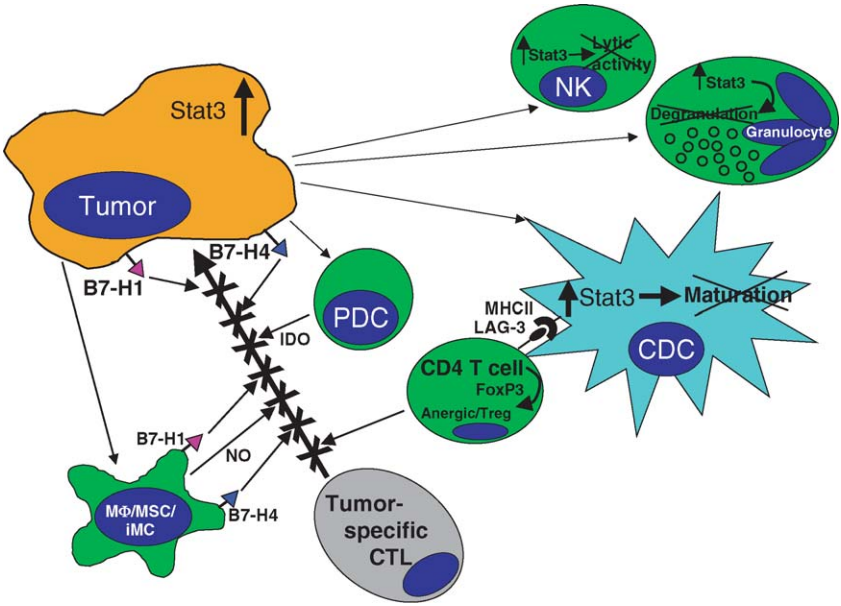

Figure 2 Multiple immunologic checkpoints in the tumor microenvironment. Tumors release factors that induce inhibition of both innate and adaptive antitumor immunity. *Stat3* activation in tumors, as well as Braf activation, can induce release of factors such as IL10 that induce *Stat3* signaling in NK cells, granulocytes, inhibiting their tumoricidal activity. *Stat3* is also activated within conventional dendritic cells (CDC) in the tumor, converting them to toleragenic DC, which can induce T cell anergy and possibly regulatory T cells (Treg). Plasmacytoid DC (PDC) or PDC-related cells in the tumor microenvironment upregulate indoleamine 2,3-dioxygenase (IDO), an enzyme that metabolizes tryptophan. T cells are very sensitive to tryptophan depletion. Tumors can express coinhibitory B7 family members, such as B7-H1 and B7-H4, which downregulate T cell activation and/or cytolytic activity. They can also induce B7-H1 and B7-H4 expression on tumor associated macrophages (TAM). Related immature myeloid cells or myeloid suppressor cells can further inhibit antitumor T cells via production of NO by the enzyme arginase.

best-studied oncogenic signaling pathway to downmodulate antitumor immune responses is the *Stat3* pathway. *Stat3* is commonly constitutively activated in diverse cancers of both hematopoietic and epithelial origin. Constitutively activated *Stat3* enhances tumor cell proliferation and prevents apoptosis. Indeed, it was found that constitutive *Stat3* activity in tumors negatively regulates inflammation, DC activity and T-cell immunity. Constitutive activation of *Stat3* in tumor cells has been shown to upregulate cell cycle regulatory, proangiogenic and antiapoptotic genes critical to the transformation process (Bowman *et al.*, 2001; Bromberg *et al.*, 1999; Niu *et al.*, 2002; Turkson *et al.*,

1998). As described previously, successful development of invasive, metastatic cancer would require the modulation of genes in a manner that inhibits activation of both innate and adaptive elements of the immune surveillance system. The *Stat3* signaling pathway in tumor cells appears to accomplish this both by inhibiting the production of proinflammatory danger signals and by inducing expression of factors that inhibit functional maturation of dendritic cells (DCs) and other tumor infiltrating hematopoietically derived cells responsible for innate immunity. Blockade of constitutive *Stat3* signaling in tumor cells resulted in the dramatic upregulation of proinflammatory cytokine genes, such as *TNF-α* and *IFN-β*. Proinflammatory chemokine genes, such as *IP-10* and *RANTES*, were also upregulated. These cytokines begin to be produced without any exogenous inductive stimuli when *Stat3* was inhibited, indicating that *Stat3* signaling restrains a natural propensity of tumors to produce these molecules (Wang *et al.*, 2004). Conversely, induction of *Stat3* signaling in 3T3 cells inhibited their production of proinflammatory cytokines and chemokines in response of TLR agonists such as LPS.

It has also been shown that elements of the tumor microenvironment can promote immune tolerance, at least in part, by bone marrow-derived DCs (Hawiger *et al.*, 2001). Indeed, DCs are immature and functionally impaired in both cancer patients and tumor-bearing animals (Almand *et al.*, 2000; Vicari *et al.*, 2002). Dysfunction of DCs in tumor-bearing hosts may be due to the lack of "danger" signals necessary for DC activation together with factors in the tumor milieu inhibiting functional maturation of DCs. *Stat3* activity additionally was found to promote the production of multiple factors, among them VEGF and IL-10, that activate *Stat3* signaling and inhibiting functional DC maturation in culture (Wang *et al.*, 2004). Inhibition of *Stat3* activity in DC cell progenitors has also been shown to reduce accumulation of immature DCs in the tumor microenvironment (Nefedova *et al.*, 2004). Tumors do not invent their own physiology but rather, dysregulate normal physiologic mechanisms to suit their own purposes. The *Stat3* pathway in tumors may represent a dysregulated wound healing response. It is well established that wounding, which causes disruption of cell–cell interactions and tissue architecture, induces release of proinflammatory cytokines. In fact, *Stat3* activation has been shown to be critical to wound healing (Sano *et al.*, 1999). Furthermore, defective wound healing in mice with selective disruption of *Stat3* in keratinocytes was associated with increased inflammatory infiltrates at wound sites. Thus, as with other aspects of cancer biology, the ability of *Stat3* activation to evade the immune system likely reflects a natural function for *Stat3* in normal physiology.

In addition to its role in tumor cells, *Stat3* activation in tumor infiltrating hematopoietic elements appears to be a major checkpoint for innate and

adaptive antitumor responses (Kortylewski *et al.*, 2005). The role of hematopoietic *Stat3* signaling was investigated by staining for phospho-*Stat3* in tumor infiltrating cells as well as inducible hematopoietic knockout of *Stat3*. Analysis of phospho-*Stat3* by flow cytometry reveals that *Stat3* is also constitutively activated in tumor-infiltrating NK cells and neutrophils. Induction of *Stat3* deletion considerably increased the number of splenic granulocytic lineage cells and reduced *Stat3* signaling in neutrophils and enhanced their cytolytic activity against target tumor cells. Furthermore, tumor-infiltrating NK cells from hematopoietically *Stat3* ablated mice demonstrated strongly enhanced cytolytic activity compared to those derived from tumor-free control mice. Many tumor-associated factors, including IL-10, VEGF, and IL-6, are activators of *Stat3*, and IL-10 is abundantly produced by tumor-associated macrophages in many different tumors (Halak *et al.*, 1999). The findings of increased function of purified *Stat3*$^{-/-}$ NK cells and neutrophils from tumor-bearing mice together with an increase in *Stat3* activity directly within these populations in tumor indicates that the role of *Stat3* signaling in downregulating function of these cell types is at least in part, cell intrinsic.

The inhibition of innate responses and DC maturation mediated by *Stat3* pathway activation in hematopoietic cells infiltrating tumors further contributes to the failure to prime tumor-specific T cells. Thus, T cells from hematopoietically *Stat3* ablated mice were able to mount stronger responses against tumor antigens than their *Stat3*$^{+/+}$ counterparts. This is associated with a considerably higher infiltration by T lymphocytes into tumor tissues from the hematopoietically *Stat3* ablated mice as well as decreases in the number of tumor infiltrating regulatory T cells (see later). These broad ranging inhibitory effects of *Stat3* signaling on innate and adaptive immune responses translate into *in vivo* antitumor responses. Ablating the *Stat3* alleles in hematopoietic cells before tumor challenge significantly inhibited growth of a number of tumor types whereas hematopoietic ablation of *Stat3* after tumor establishment resulted in either slowed growth or outright regression of tumors.

The central role of *Stat3* signaling in both tumors and hematopoietic cells as an immunologic checkpoint defines it as an interesting target for antagonism to enhance antitumor immunity. Recently, a small molecule *Stat3* inhibitor, CPA-7, was identified. CPA-7 disrupts *Stat3* DNA-binding activity, which is followed, within hours, by a reduction of phospho-*Stat3* protein in the treated cells *in vitro* (Turkson *et al.*, 2005). Tumor-infiltrating DCs from mice receiving CPA-7 displayed considerably reduced phospho-*Stat3* compared to vehicle-treated mice. CPA-7 treatment *in vivo* also led to a significant growth inhibition of established tumors that was both T cell and NK cell dependent.

Similar results were observed using a Jak2/*Stat3* inhibitor, JSI-124 (Nefedova *et al.*, 2005).

In addition to its role in inhibiting the activation and effector function of DC, granulocytes, and NK cells in the tumor microenvironment, *Stat3* signaling has also been reported to play a role in guiding immature myeloid cells (iMC) to differentiate into myeloid suppressor cells (MSC) rather than DC with APC activity. Immature myeloid cells (Kusmartsev and Gabrilovich, 2005; Young *et al.*, 2001) and MSC (Bronte *et al.*, 2000, 2003; Mazzoni *et al.*, 2002; Zea *et al.*, 2005) represent a cadre of myeloid cell types, including tumor-associated macrophages (TAM), that share the common feature of inhibiting both the priming and effector function of tumor-reactive T cells. It is still not clear whether these myeloid cell types represent distinct lineages or different states of the same general immune inhibitory cell subset. In mice, iMC and MSC are characterized by coexpression of CD11b (considered a macrophage marker) and Gr1 (considered a granulocyte marker) while expressing low or no MHC class II or the CD86 costimulatory molecule. In humans, they are defined as $CD33^+$ but lacking markers of mature macrophages, DCs or granulocytes and are DR^-. A number of molecular species reported to be produced by tumors tend to drive iMC/MSC accumulation. These include IL-6, CSF-1, IL-10, and gangliosides. IL-6 and IL-10 are potent inducers of *Stat3* signaling. Another cytokine reported to induce iMC/MSC accumulation is GM-CSF (Serafini *et al.*, 2004). This finding is somewhat paradoxical, since GM-CSF is a critical inducer of DC differentiation and GM-CSF transduced tumor vaccines enhance antitumor T-cell immunity via accumulation of DCs at the vaccine site followed by increased DC numbers in vaccine draining LN. It appears that the paradox is solved based on levels of GM-CSF. High-local levels drive DC differentiation at the vaccine site whereas chronic production of low levels of GM-CSF can promote iMC/MSC accumulation. GM-CSF transduced vaccines that produce extremely high GM-CSF levels can induce iMC/MSC accumulation at distant sites (i.e., spleen and LNs) because they release enough GM-CSF systemically to drive iMC/MSC accumulation.

A number of mechanisms have been proposed to explain how iMC/MSC inhibit T-cell responses. Most include the production of ROS and/or RNS. NO production by iMC/MSC as a result of arginase activity, which is high in these cells, has been well documented and inhibition of this pathway with a number of drugs can mitigate the inhibitory effects of iMC/MSC. ROS, including H_2O_2, have been reported to block T-cell function associated with the down-modulation of the ζ chain of the TCR signaling complex (Schmielau and Finn, 2001); a phenomenon well recognized in T cells from cancer patients and associated with generalized T-cell unresponsiveness.

Another mediator of T-cell unresponsiveness associated with cancer is the production of indolamine 2,3-dioxygenase (IDO) (Munn *et al.*, 2002). IDO appears to be produced by DCs either within tumors or in tumor draining LN. Interestingly, IDO in DCs has been reported to be induced via backward signaling by B7-1/2 upon ligation with CTLA-4 (Baban *et al.*, 2005; Mellor *et al.*, 2004). Apparently, the major IDO producing DC subset is either a plasmacytoid DC (PDC) or a PDC-related cell that is $B220^+$ (Munn *et al.*, 2004). IDO appears to inhibit T-cell responses through catabolism of tryptophan. Activated T cells are highly dependent on tryptophan and are therefore sensitive to tryptophan depletion. Thus, Munn and Mellor have proposed a bystander mechanism, whereby DCs in the local environment deplete tryptophan via IDO upregulation, thereby inducing metabolic apoptosis in locally activated T cells.

Another inhibitory molecule produced by many cell types that has been implicated in blunting antitumor immune responses is transforming growth factor beta (TGF-β), which is produced by a variety of cell types, including tumor cells, and which has pleiotropic physiological effects. For most normal epithelial cells, TGF-β is a potent inhibitor of cell proliferation, causing cell cycle arrest in the G1 stage (Blobe *et al.*, 2000). In many cancer cells, however, mutations in the TGF-β pathway confer resistance to cell cycle inhibition, allowing uncontrolled proliferation. Additionally, in cancer cells, the production of TGF-β is increased, and may contribute to invasion by promoting the activity of matrix metaloproteinases. *In vivo*, TGF-β directly stimulates angiogenesis; this stimulation can be blocked by anti-TGF-β antibodies (Pepper, 1997). A bimodal role of TGF-β in cancer has been verified in a transgenic animal model using a keratinocyte-targeted overexpression (Cui *et al.*, 1996). Initially, these animals are resistant to the development of early-stage or benign skin tumors. However, once tumors form, they progress rapidly to a more aggressive spindle-cell phenotype. While this clear bimodal pattern of activity is more difficult to identify in a clinical setting—it should be noted that elevated serum TGF-β levels are associated with poor prognosis in a number of malignancies, including prostate cancer (Shariat *et al.*, 2001), lung cancer (Hasegawa *et al.*, 2001), gastric cancer (Saito *et al.*, 1999) and bladder cancer (Shariat *et al.*, 2001).

From an immunological perspective, TGF-β possesses broadly immunosuppressive properties and TGF-β knockout mice develop widespread inflammatory pathology and corresponding accelerated mortality (Letterio and Roberts, 1998). Interestingly, a majority of these effects seem to be T-cell mediated, as targeted disruption of T cell TGF-β signaling also results a similar autoimmune phenotype (Gorelik and Flavell, 2000). Recent experiments by Chen *et al.* (2005) rather convincingly demonstrated a role for TGF-β in Treg mediated

suppression of CD8 T cell antitumor responses. In these experiments, adoptive transfer of $CD4^+$ $CD25^+$ regulatory T cells inhibited an antitumor CD8 T-cell effector response, and that this inhibition was ameliorated when the CD8 T cells came from animals with a dominant negative TGF-β1 receptor. In an analogous manner, Zhang *et al.* (2005) performed *ex vivo* transduction of CD8 T cells with a dominant negative TGF-β receptor and then adoptively transferred these cells back into mice with progressive, endogenous prostate tumors. Treated animals showed tumor regression and enhanced survival, demonstrating that TGF-β mediated attenuation of endogenous CD8 T-cell function might be important in tumor progression. Recent data from Ahmadzadeh *et al.* extend these findings to an *in vitro* examination of CD8 T cells from patients who received a gp100 targeted vaccine for melanoma (Ahmadzadeh and Rosenberg, 2005). These cells showed impaired effector function when TGF-β was present in the initial antigen activation cultures, and this attenuation was also noted in melanoma specific T-cell clones. Taken together, these data suggest that either antibody mediated (Lucas *et al.*, 1990), or pharmacological (Callahan *et al.*, 2002; Laping *et al.*, 2002) blockade of TGF-β may prove to be an important component of combinatorial immunotherapy strategies.

4. Disabled Antigen Presenting Cells

One of the unresolved issues in the study of tumor immune evasion relates to the mechanisms by which tumors induce antigen-specific T-cell tolerance. While the many mechanisms described earlier, including *Stat3* signaling dependent mechanisms, IDO, ROS, RNS, TGF-β, etc., clearly inhibit priming of T-cell responses and/or tumor killing by activated effector T cells, it remains to be definitively determined which processes actively induce antigen-specific T-cell tolerance that has been documented in transgenic models. Self-tolerance induction for peripheral tissue antigens is now thought to involve specific presentation of tissue-specific antigens to mature T cells in the absence of appropriate costimulatory signals. Similar mechanisms are likely operative in the case of tumor-induced tolerance. Originally, the relevant costimulatory signals were envisioned to be provided by B7 family costimulatory molecules expressed by DCs (Schwartz, 1992). It is now becoming clear that additional proinflammatory cytokines, such as interferons, IL-12, TNF, etc., are critical in the distinction between effector T-cell induction and tolerance induction.

An emerging concept is that immature or not fully matured DCs are critical in presenting self-antigens to induce T-cell tolerance in the absence of TLR-mediated danger signals associated with infection (Bonifaz *et al.*, 2002; Hawiger *et al.*, 2001; Steinman *et al.*, 2003). Unquestionably, DCs found within the tumor microenvironment have a relatively immature, inactivated

phenotype characterized by low levels of proinflammatory cytokine production, CD86, and surface MHC class II expression. As described earlier, a major inhibitory signaling pathway induced in tumor infiltrating DC is the *Stat3* pathway which, when activated, strongly antagonizes TLR and CD40-mediated DC activation. As mentioned, tumor-derived factors, such as IL-10, IL-6, and VEGF (in part induced by *Stat3* signaling in the tumor cell), can induce *Stat3* activation in DCs. Activated *Stat3* in DCs may directly inhibit NFκB signaling either at the level of IKK or further downstream within the nucleus. Additional oncogenic signaling in tumors may contribute to inhibition of activation of DCs in the tumor microenvironment. Recently, constitutive BRAF signaling in melanoma cells (due to an activating mutation found in over 60% of melanomas) has been shown to induce release of factors that inhibit DC activation (Sumimoto *et al.*, 2006). These immature "activation-inhibited" DCs clearly represent a prime candidate for the induction of tumor-specific T-cell tolerance.

It remains an open question as to whether iMC/MSC represent a distinct intertumoral cell subset capable of presenting antigens to T cells in a toleragenic fashion (Kusmartsev *et al.*, 2004). A recent report indeed suggested that iMC loaded with antigen and adoptively transferred into mice can induce antigen-specific T-cell tolerance. Finally, it has been suggested that IDO-expressing DC can induce antigen-specific T-cell tolerance because IDO-mediated tryptophan selectively kills or inhibits proliferation of activated T cells (Munn *et al.*, 2005). According to this model, IDO-expressing DCs would present antigen to T cells inducing activation followed by activation-associated cell death mediated by depletion of local tryptophan stores by the IDO in the presenting DCs. As described later, regulatory T cells play an additional important role in induction or maintenance of tumor antigen-specific T-cell tolerance. Whether Treg cells mediate T-cell tolerance independently from immature or toleragenic APCs or whether the two mechanisms are completely interrelated (i.e., toleragenic DCs inducing a Treg phenotype among antigen-specific T cells and antigen-specific Treg cells acting upon DCs to enhance their toleragenic capacity) remains to be definitely determined.

5. CD4 T-Cell Tolerance

The immune system has evolved multiple mechanisms to avoid a CD4-mediated autoimmune response (Schwartz, 1999). Perhaps the most well studied of these is the deletion of autoreactive T cells at the CD4/CD8 double positive stage in the thymus (Blackman *et al.*, 1992; Hugo *et al.*, 1991; Kappler *et al.*, 1987; MacDonald *et al.*, 1988; Surh and Sprent, 1994). In an animal model, Bogen *et al.* have provided convincing evidence that such events may

occur in the progression of myeloma (Bogen *et al.*, 2000; Lauritzsen *et al.*, 1998). In an elegant system, mice were engineered to express a T-cell receptor specific for a myeloma protein. These TCR transgenic mice are quite adept at responding to and rejecting small implanted tumors (Bogen *et al.*, 1995). However, when the primary implant becomes larger than 0.5 cm, the tumors become increasingly progressive. Analysis of thymic T cells in these mice revealed deletion at the double positive stage of ontogeny, strongly resembling that seen in classical thymic deletion models (Lauritzsen *et al.*, 1998). A second, perhaps more subtle, tolerance mechanism involves anergy, a state of antigen-specific unresponsiveness, which occurs when the T-cell receptor engages the MHC antigen-peptide complex in the absence of appropriate costimulatory signals (Bretschen, 1970; Quill and Schwartz, 1987; Schwartz *et al.*, 1989). Anergy as a mechanism for T-cell tolerance to tumors has been demonstrated in several model systems (Mizoguchi *et al.*, 1992; Staveley-O'Carroll *et al.*, 1998). In one such system, A20 lymphoma cells were stably transfected with the influenza hemagglutinin peptide (HA) to provide a unique tumor antigen. When HA-specific T cells are adoptively transferred to mice bearing progressive A20 (HA) lymphomas, the result was not the vigorous immune rejection one might have predicted (and hoped for). In fact, the transferred-specific T cells were rapidly rendered anergic, incapable of proliferating *in vitro* to specific antigen. Perhaps more importantly, A20 (HA) tumors progressed at an identical rate whether the mice received specific adoptively transferred T cells.

Antigen-specific T priming of T cells can be either direct (i.e., via Class I-antigen complexes expressed on the target cell type) or indirect (i.e., via complexes presented on bone marrow-derived professional antigen presenting cells) (Adler *et al.*, 1998). For most tumor types, direct presentation appears to be minimal whereas cross-presentation represents the dominant presentation pathway (Huang *et al.*, 1994). However, a number of groups have noted that this cross priming pathway is not especially efficient, leading to circumstances in which small, evolving tumors are mostly "ignored" by the host immune system (Ochsenbein *et al.*, 1999). Tolerance would, therefore, remain intact until tumors become metastatic to draining lymph nodes, at which time the host could potentially mount an antitumor response (Ochsenbein *et al.*, 2005).

6. Coinhibition

While the Bretscher and Cohn two signal model of T-cell activation has gained wide acceptance and is backed by copious experimental data, recent studies have suggested that this model may be inadequate under certain circumstances, and that T-cell activation and effector function may ultimately be

controlled by a balance between costimulatory molecules (such as CD28) and coinhibitory molecules (Chen *et al.*, 2004). The canonical coinhibitory pathway is that mediated by the interaction between CTLA-4 (on T cells) and its ligands (B7-1 and B7-2) on antigen presenting cells. CTLA-4 is not robustly expressed on naïve T cells, but is rapidly induced after T-cell activation, and, as is the case for TGF-β, CTLA-4 knockout mice develop lymphoproliferative autoimmunity, indicating a tonic role for CTLA-4—B7-1/B7-2 interactions in the prevention of autoimmunity (Waterhouse *et al.*, 1995). Based on these data, CTLA-4 blockade would be expected to function mostly during T cell priming events, facilitating or enhancing an immune response. In a number of murine systems, CTLA-4 blockade exerts a pronounced antitumor effect (Chambers *et al.*, 2001), generating enthusiasm for translating these observations to the clinic. Clinical trials to date have primarily evaluated a single agent approach, using a human anti-CTLA-4 monoclonal blocking antibody (MDX010, Medarex, Inc.). While some clinical efficacy has been demonstrated at higher antibody doses (Phan *et al.*, 2003), a number of autoimmune side effects have been observed, including inflammatory bowel pathology. Interestingly, this pathology bears a remarkable resemblance to that seen in the CTLA-4 knockout mice, highlighting the utility of preclinical models in the study of immunotherapy.

A second coinhibitory molecule on T cells is PD1 (programmed death 1), a T cell surface molecule originally discovered in a T-cell hybridoma undergoing apoptosis (Ishida *et al.*, 1992). Further studies of PD1 identified expression on activated, but not naïve T and B cells, in addition to potential overexpression in anergized CD4 T cells (Hatachi *et al.*, 2003; Lechner *et al.*, 2001). Recent data show that PD1 is also expressed on the surface of certain CD8 T cells, where it serves as a marker for T cells that have been "exhausted" by exposure to persistent viral antigen *in vivo* (R. Ahmed, unpublished observations). As is the case for CTLA-4 and TGF-β, PD1 knockout mice develop strain-dependent autoimmune disease (Nishimura *et al.*, 1999, 2001). In murine models of experimental autoimmune encephalomyelitis (EAE) and diabetes, anti-PD1 antagonist antibodies enhance disease progression (Ansari *et al.*, 2003; Salama *et al.*, 2003). There are currently two known ligands for PD1, B7-H1 (also known as PD-L1) and B7-DC (also known as PD-L2). These ligands have very different tissue distribution patterns, wit B7-DC expression is primarily confined to DCs and macrophages (Tseng *et al.*, 2001). B7-H1 mRNA is widely expressed, but cell surface protein is not detectable in normal tissues other than a subset of macrophages (Dong *et al.*, 1999). Interestingly, B7-H1 expression can be detected in a number of tumor types (Dong *et al.*, 2002), and engagement of PD1 by tumor associated B7-H1 promotes CD8 T-cell apoptosis. Clinically, it has been reported that B7-H1 expression is correlated with poor prognosis in renal cell carcinoma (Thompson *et al.*, 2004). Thus, it seems that PD1/B7-H1

interactions mediate a potent and specific immunoregulatory effect, preventing activated and trafficking CD8 T cells from lysing their targets *in vivo*. In recent data, this observation has been confirmed in murine tumor models, where blockade of either PD1 or of the PD1 ligand B7-H1 potentiates an antitumor immune response (Hirano *et al.*, 2005). In contrast, the molecular role of B7-DC ligation in an immune response is complex (Chen *et al.*, 2004), and under some circumstances ligation of B7-DC on antigen presenting cells appears to potentiate a costimulatory interaction with T cells (Nguyen *et al.*, 2002a,b; Shin *et al.*, 2003). Clearly, further research is required to determine the relative roles of these two PD1 ligands in human tumor immunology. However, all of the currently available data do strongly support a role for PD1 blockade in the potentiation of an antitumor immune response.

Murphy and colleagues, have recently described a new member of the coinhibitor group of molecules, which they coined BTLA (B and T lymphocyte attenuator) (Watanabe *et al.*, 2003). The BTLA is expressed on activated T cells as well as B cells and DCs. Interestingly, like PD1, BTLA also appears to be relatively upregulated on anergic CD4 T cells (Hurchla *et al.*, 2005). While CTLA-4 and PD1 bind to B7 family members, the ligand for BTLA has been identified as HVEM (herpesvirus entry mediator) (Sedy *et al.*, 2005), which is a member of the TNFR superfamily (Croft *et al.*, 2005). This interaction is interesting and represents a unique mechanism for potential crosstalk between the CD28-B7 family and the TNFR-TNF family. Dissecting the overall immune outcome mediated by interactions between BTLA and HVEM is complicated by the fact that HVEM also binds to two additional ligands (LIGHT and secreted lymphotoxin) (Croft *et al.*, 2005). Based on this complexity, a number of signaling models are possible, all of which await further experimental confirmation.

7. Regulatory T Cells

Aside from the burgeoning families of coinhibitory and costimulatory molecules, a great deal of interest in both tumor immunity and infectious immunity has focused on regulatory T cells (Treg) (von Boehmer, 2005). This interest dates back to the observations of Sakaguchi *et al.*, who created a renaissance in T-cell suppression by demonstrating that CD4 T cells that were $CD25^+$ could prevent the development of autoimmunity mediated by neonatal thymectomy (Sakaguchi *et al.*, 1995). The regulatory potential of thymic $CD4^+CD25^+$ T cells was subsequently confirmed in a number of autoimmunity, infection, and tumor models (Shevach, 2004) (Fig. 3). Perhaps the most convincing data concerning a role for $CD4^+CD25^+$ regulatory T cells in human cancer come from the recent work of Curiel *et al.*, who showed that the presence of such regulatory T cells in ascites fluid from women with advanced ovarian cancer

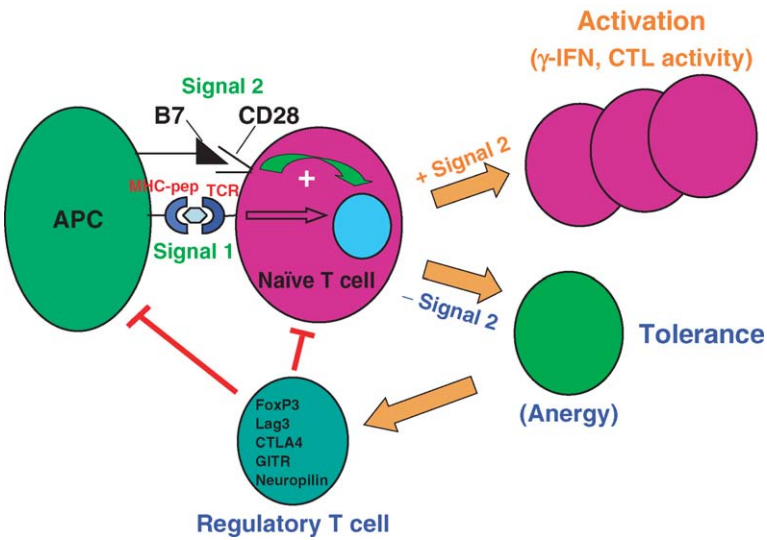

Figure 3 Regulatory T cells are an important inhibitor of antitumor immunity. T cell activation in the absence of appropriate costimulatory signals leads to T cell anergy and generation of induced regulatory T cells (Treg). Treg, characterized by the FoxP3 transcription factor, upregulate a number of cell membrane molecules, including Lag3, CTLA4, GITR, and neuropilin. Treg can inhibit effector T cell activation and function via T-T inhibition or inhibition of antigen presenting cells.

was associated with decreased survival (Curiel *et al.*, 2004). In addition, these cells were shown to exhibit regulatory function in *ex vivo* Treg assays. Subsequently, tumor-associated Treg have been identified in lung, pancreatic, ovarian, and breast cancer specimens (Liyanage *et al.*, 2002).

There is presently no single cell surface marker that uniquely identifies Treg. As has been described, CD25 (the IL-2 receptor alpha chain) marks activated as well as regulatory T cells, and thus CD4 CD25 T cells contain a mixture of activated as well as regulatory T cells. Other potential Treg markers include the glucocorticoid-induced TNF receptor (GITR) (McHugh *et al.*, 2002), neuropilin (Bruder *et al.*, 2004), and lymphocyte activation gene-3 (Lag-3) (Huang *et al.*, 2004). This lack of a clear cell surface marker not only complicates the study of Treg in model systems but also makes it difficult to envision Treg specific intervention strategies. Nonetheless, absolute specificity may not be completely necessary; in many systems, systemic depletion of CD4 CD25 T cells by monoclonal antibodies (PC-61 in the mouse) or by a CD25 targeted toxin (Ontac) clearly augments a specific immune response (Terabe and Berzofsky, 2004). Another interesting methodology for functionally targeting Treg stems from seminal observations made by Robert North in the

late 1970s, who showed that low doses of the common chemotherapy agent cyclophosphamide appeared to selectively deplete cells with regulatory activity (North, 1982). This observation has been widely confirmed in the clinical setting (Berd *et al.*, 1982, 1986), and recent studies using murine models of prostate and breast cancer have helped to define the mechanism of this effect (Machiels *et al.*, 2001; Nigam *et al.*, 1998). Perhaps the most intriguing data regarding the effect of cyclophosphamide in a tumor model come from Ercolini *et al.*, who showed that immunoregulatory doses of cyclophosphamide seemed to selectively unmask a high affinity population of tumor-specific CD8 T cells (Ercolini *et al.*, 2005). Based on the relatively low toxicity of these regimens (no lymphopenia is induced), there seems little reason not to consider the addition of low-dose cyclophosphamide to antitumor immunotherapy approaches.

8. CD8 T-Cell Dysfunction

Because tumors have likely been present for years before they are clinically detectable, endogenous tumor-specific CD8 T cells have been exposed to tumor antigens for protracted time periods. This situation of persistent antigen has been investigated using a number of chronic viral models (Zajac *et al.*, 1998). Data from such systems are consistent in showing impaired CD8 T-cell function under these conditions. Interestingly, antigen-specific CD8 T cells do not appear to be specifically deleted, but rather enter a state where they cannot transition between an effector memory ($CD62L^{low}$) and central memory ($CD62L^{high}$) phenotype. This phenomenon has been termed "exhaustion" and may play a role in impaired CD8 T-cell response to persistent tumor antigens as well. In a way, the phenomenon of CD8 T-cell exhaustion is actually encouraging from the perspective of immunotherapy, suggesting that tumor-specific CD8 T cells may be present and partially primed, even in the tumor-bearing host. The removal of persistent antigen can have profound effects under such conditions—den Boer *et al.* recently showed that CD8 T cells isolated from tumor bearing hosts could regain function in tumor free animals; functionally mediating protection from tumor challenge (den Boer *et al.*, 2004). These findings echo seminal experiments performed by Ramsdell and Fowlkes, who, over two decades ago showed that elimination of persistent antigen could restore T-cell function *in vivo* (Ramsdell and Fowlkes, 1992). Translating these observations to the clinical setting is not straightforward but suggests that immunotherapy approaches may prove most efficacious in the setting of minimal residual disease (Finn, 2003). For certain tumor types, i.e. chemosensitive small cell lung cancer (SCLC) or hormone responsive prostate cancer, a minimal residual disease state is routinely encountered following standard therapy, representing an attractive patient population for clinical trials.

Animal models of insulinoma have shed some light on the response of tumor-specific CD8 T cells *in vivo* (Lyman *et al.*, 2004; Nguyen *et al.*, 2002a,b). In one such model, Nguyen *et al.* showed that adoptively transferred tumor-specific CD8 T cells differentiated into an effector phenotype, and mediated a modicum of protection *in vivo* (Nguyen *et al.*, 2002a,b). Using a different CD8 T-cell transgenic donor, Lyman *et al.* reported similar findings, that adoptively transferred CD8 T cells proliferated and acquired cytolytic activity *in vivo* (Lyman *et al.*, 2004). Together these data suggest that CD8 T-cell tolerance to tumors may not be as profound as that of CD4 T cells, and that provision of adequate CD4 T-cell help to low affinity tumor-specific T cells may be sufficient to overcome a relative of *in vivo* cytolytic efficacy (Lyman *et al.*, 2004).

When CD8 T cells are activated by antigen presenting cells in a manner that facilitates the acquisition of full effector function, they lyse target cells by a combination of granzyme and perforin-mediated mechanisms. The antigen presenting cells themselves appear to be far less susceptible to CD8 T-cell mediated lysis. One of the mechanisms for this protection has recently been elucidated and stems from the observation that activated antigen presentation cells express a serine protease inhibitor (SPI)-6, a member of the serpin family that specifically inactivates granzyme B and blocks CTL-induced apoptosis (Medema *et al.*, 2001). Surprisingly, recent data show that several colon carcinoma cell lines resistant to CTL-mediated killing express SPI-6 in addition to an additional required serpin known as SPI-CI (serine protease inhibitor involved in cytotoxicity inhibition) (Bots *et al.*, 2005). These data are relatively recent, but they suggest yet another pathway by which tumors may resist killing by activated CD8 T cells. Future studies examining the relative expression of serpins in various tumor types should elucidate the magnitude to which this mechanism inhibits an antitumor immune response in the clinical setting.

9. Summary

While immunologic checkpoints that potentially block antitumor immune responses are becoming well defined at the molecular level, the relative importance of circumventing any individual checkpoint *in vivo* remains to be determined. There has been a tendency to focus upon model systems in which a single intervention renders a previously ineffective immunotherapy approach markedly efficacious. Over the next several years, it will be of primary importance to begin to develop a sense of which checkpoint intervention approaches are of most crucial, both in laboratory models as well as in the clinical situation. It is particularly challenging to clinically develop combinatorial strategies in which blockade of multiple checkpoints is evaluated. For example, if CTLA-4 is blocked, does inhibition of Treg with low-dose cyclophosphamide still have

an effect? Or, if TGF-β is neutralized with a blocking antibody or pharmacological inhibitor, would Treg depletion with an anti-CD25 targeted monoclonal antibody be redundant? As Phase II clinical trials to evaluate combination immunotherapy approaches are difficult to design and costly to implement, strong preclinical data from multiple model systems would go a long way toward guiding clinical development. Regardless of the eventual therapeutic development pathway involved, the plethora of barriers present in the tumor-bearing host makes it unlikely that single interventions (i.e., vaccination alone) or blockade of a single checkpoint would achieve a meaningful survival or quality of life benefit in patients with advanced metastatic cancer. Rather, in a manner analogous to combination chemotherapy, it seems that rational combinations targeting multiple nonoverlapping checkpoints will be required. For example, multiple datasets concur that immunological approaches are most successful in the setting of minimal residual disease, or perhaps even in the adjuvant setting, where radiologically detectable tumor may not be present. Thus, the first part of a combination strategy might be to minimize tumor antigen load using hormonal therapy, surgery, radiation, or conventional chemotherapy. Next, checkpoints that affect the processing and presentation of tumor antigen—such as *Stat3* or TGF-β might be blocked. Following this, specific immunization might be employed, using one of several immunization approaches currently under Phase III evaluation. As T cells respond to vaccination, checkpoints like CTLA-4, which affect the priming phase of a T-cell response, might be blocked. Finally, those checkpoints that affect CD8 T-cell function in the tumor environment, such as B7-H1, might be targeted. While such a sequential, combinatorial approach may seem hopelessly complex to a laboratory-based immunologist, far more involved clinical regimens, employing multiple highly toxic chemotherapy regimens in sequence, are utilized every day in the treatment of human cancer. Immunologic strategies may need to approach this level of complexity in order to succeed.

References

Adler, A. J., Marsh, D. W., Yochum, G. S., Guzzo, J. L., Nigam, A., Nelson, W. G., and Pardoll, D. M. (1998). CD4+ T cell tolerance to parenchymal self-antigens requires presentation by bone marrow-derived antigen-presenting cells. *J. Exp. Med.* **187**(10), 1555–1564.

Ahmadzadeh, M., and Rosenberg, S. A. (2005). TGF-beta 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. *J. Immunol.* **174**(9), 5215–5223.

Almand, B., Resser, J. R., Lindman, B., Nadaf, S., Clark, J. I., Kwon, E. D., Carbone, D. P., and Gabrilovich, D. I. (2000). Clinical significance of defective dendritic cell differentiation in cancer. *Clin. Cancer Res.* **6**(5), 1755–1766.

Alpan, R. S., Zhang, M., and Pardee, A. B. (1996). Cell cycle-dependent expression of TAP1, TAP2, and HLA-B27 messenger RNAs in a human breast cancer cell line. *Cancer Res.* **56**(19), 4358–4361.

Ansari, M. J., Salama, A. D., Chitnis, T., Smith, R. N., Yagita, H., Akiba, H., Yamazaki, T., Azuma, M., Iwai, H., Khoury, S. J., Auchincloss, H., Jr., and Sayegh, M. H. (2003). The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J. Exp. Med.* **198**(1), 63–69.

Baban, B., Hansen, A. M., Chandler, P. R., Manlapat, A., Bingaman, A., Kahler, D. J., Munn, D. H., and Mellor, A. L. (2005). A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type I IFN signaling following B7 ligation. *Int. Immunol.* **17**(7), 909–919.

Bakkenist, C. J., and Kastan, M. B. (2004). Initiating cellular stress responses. *Cell* **118**(1), 9–17.

Benitez, R., Godelaine, D., Lopez-Nevot, M. A., Brasseur, F., Jimenez, P., Marchand, M., Oliva, M. R., van Baren, N., Cabrera, T., Andry, G., Landry, C., Ruiz-Cabello, F., *et al.* (1998). Mutations of the beta2-microglobulin gene result in a lack of HLA class I molecules on melanoma cells of two patients immunized with MAGE peptides. *Tissue Antigens* **52**(6), 520–529.

Berd, D., Mastrangelo, M. J., Engstrom, P. F., Paul, A., and Maguire, H. (1982). Augmentation of the human immune response by cyclophosphamide. *Cancer Res.* **42**(11), 4862–4866.

Berd, D., Maguire, H. C., Jr., and Mastrangelo, M. J. (1986). Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. *Cancer Res.* **46**(5), 2572–2577.

Blackman, M. A., Lund, F. E., Surman, S., Corley, R. B., and Woodland, D. L. (1992). Major histocompatibility complex-restricted recognition of retroviral superantigens by V beta 17+ T cells. *J. Exp. Med.* **176**(1), 275–280.

Blanchet, O., Bourge, J. F., Zinszner, H., Israel, A., Kourilsky, P., Dausset, J., Degos, L., and Paul, P. (1992). Altered binding of regulatory factors to HLA class I enhancer sequence in human tumor cell lines lacking class I antigen expression. *Proc. Natl. Acad. Sci. USA* **89**(8), 3488–3492.

Blobe, G. C., Schiemann, W. P., and Lodish, H. F. (2000). Role of transforming growth factor beta in human disease. *N. Engl. J. Med.* **342**(18), 1350–1358.

Bogen, B., Munthe, L., Sollien, A., Hofgaard, P., Omholt, H., Dagnaes, F., Dembic, Z., and Lauritzsen, G. F. (1995). Naive CD4+ T cells confer idiotype-specific tumor resistance in the absence of antibodies. *Eur. J. Immunol.* **25**(11), 3079–3086.

Bogen, B., Schenck, K., Munthe, L. A., and Dembic, Z. (2000). Deletion of idiotype (Id)-specific T cells in multiple myeloma. *Acta Oncol.* **39**(7), 783–788.

Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M. C., and Steinman, R. M. (2002). Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J. Exp. Med.* **196**(12), 1627–1638.

Bots, M., Kolfschoten, I. G., Bres, S. A., Rademaker, M. T., de Roo, G. M., Kruse, M., Franken, K. L., Hahne, M., Froelich, C. J., Melief, C. J., Offringa, R., and Medema, J. P. (2005). SPI-CI and SPI-6 cooperate in the protection from effector cell-mediated cytotoxicity. *Blood* **105**(3), 1153–1161.

Bowman, T., Broome, M. A., Sinibaldi, D., Wharton, W., Pledger, W. J., Sedivy, J. M., Irby, R., Yeatman, T., Courtneidge, S. A., and Jove, R. (2001). *Stat3*-mediated Myc expression is required for Src transformation and PDGF-induced mitogenesis. *Proc. Natl. Acad. Sci. USA* **98**(13), 7319–7324.

Bretschen, P., and Cohn, M. (1970). A theory of self-nonself discrimination. *Science* **169,** 1042–1049.

Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., and Darnell, Bronte, J. E., Jr. (1999). *Stat3* as an oncogene. *Cell* **98**(3), 295–303.

Bronte, V., Apolloni, E., Cabrelle, A., Ronca, R., Serafini, P., Zamboni, P., Restifo, N. P., and Zanovello, P. (2000). Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood* **96**(12), 3838–3846.

Bronte, V., Serafini, P., De Santo, C., Marigo, I., Tosello, V., Mazzoni, A., Segal, D. M., Staib, C., Lowel, M., Sutter, G., Colombo, M. P., and Zanovello, P. (2003). IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice. *J. Immunol.* **170**(1), 270–278.

Bruder, D., Probst-Kepper, M., Westendorf, A. M., Geffers, R., Beissert, S., Loser, K., von Boehmer, H., Buer, J., and Hansen, W. (2004). Neuropilin-1: A surface marker of regulatory T cells. *Eur. J. Immunol.* **34**(3), 623–630.

Cabrera, T., Angustias, F. M., Sierra, A., Garrido, A., Herruzo, A., Escobedo, A., Fabra, A., and Garrido, F. (1996). High frequency of altered HLA class I phenotypes in invasive breast carcinomas. *Hum. Immunol.* **50**(2), 127–134.

Cabrera, T., Collado, A., Fernandez, M. A., Ferron, A., Sancho, J., Ruiz-Cabello, F., and Garrido, F. (1998). High frequency of altered HLA class I phenotypes in invasive colorectal carcinomas. *Tissue Antigens* **52**(2), 114–123.

Callahan, J. F., Burgess, J. L., Fornwald, J. A., Gaster, L. M., Harling, J. D., Harrington, F. P., Heer, J., Kwon, C., Lehr, R., Mathur, A., Olson, B. A., Weinstock, J., *et al.* (2002). Identification of novel inhibitors of the transforming growth factor beta1 (TGF-beta1) type 1 receptor (ALK5). *J. Med. Chem.* **45**(5), 999–1001.

Chambers, C. A., Kuhns, M. S., Egen, J. G., and Allison, J. P. (2001). CTLA-4-mediated inhibition in regulation of T cell responses: Mechanisms and manipulation in tumor immunotherapy. *Annu. Rev. Immunol.* **19,** 565–594.

Chen, L. (2004). Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol.* **4**(5), 336–347.

Chen, M. L., Pittet, M. J., Gorelik, L., Flavell, R. A., Weissleder, R., von Boehmer, H., and Khazaie, K. (2005). Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals *in vivo*. *Proc. Natl. Acad. Sci. USA* **102**(2), 419–424.

Clarke, B., and Chetty, R. (2002). Postmodern cancer: The role of human immunodeficiency virus in uterine cervical cancer. *Mol. Pathol.* **55**(1), 19–24.

Cormier, J. N., Abati, A., Fetsch, P., Hijazi, Y. M., Rosenberg, S. A., Marincola, F. M., and Topalian, S. L. (1998). Comparative analysis of the *in vivo* expression of tyrosinase, MART-1/Melan-A, and gp100 in metastatic melanoma lesions: Implications for immunotherapy. *J. Immunother.* **21**(1), 27–31.

Cormier, J. N., Panelli, M. C., Hackett, J. A., Bettinotti, M. P., Mixon, A., Wunderlich, J., Parker, L. L., Restifo, N. P., Ferrone, S., and Marincola, F. M. (1999). Natural variation of the expression of HLA and endogenous antigen modulates CTL recognition in an *in vitro* melanoma model. *Int. J. Cancer* **80**(5), 781–790.

Croft, M. (2005). The evolving crosstalk between co-stimulatory and co-inhibitory receptors: HVEM-BTLA. *Trends Immunol.* **26**(6), 292–294.

Cui, W., Fowlis, D. J., Bryson, S., Duffie, E., Ireland, H., Balmain, A., and Akhurst, R. J. (1996). TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* **86**(4), 531–542.

Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J. R., Zhang, L., Burow, M., Zhu, Y., Wei, S., *et al.* (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* **10**(9), 942–949.

de Vries, T. J., Trancikova, D., Ruiter, D. J., and van Muijen, G. N. (1998). High expression of immunotherapy candidate proteins gp100, MART-1, tyrosinase and TRP-1 in uveal melanoma. *Br. J. Cancer* **78**(9), 1156–1161.

den Boer, A. T., van Mierlo, G. J., Fransen, M. F., Melief, C. J., Offringa, R., and Toes, R. E. (2004). The tumoricidal activity of memory CD8+ T cells is hampered by persistent systemic antigen,

but full functional capacity is regained in an antigen-free environment. *J. Immunol.* **172**(10), 6074–6079.

Dong, H., Zhu, G., Tamada, K., and Chen, L. (1999). B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat. Med.* **5**(12), 1365–1369.

Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., Roche, P. C., Lu, J., Zhu, G., Tamada, K., Lennon, V. A., Celis, E., *et al.* (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nat. Med.* **8**(8), 793–800.

Doyle, A., Martin, W. J., Funa, K., Gazdar, A., Carney, D., Martin, S. E., Linnoila, I., Cuttitta, F., Mulshine, J., Bunn, P., and Minna, J. (1985). Markedly decreased expression of class I histocompatibility antigens, protein, and mRNA in human small-cell lung cancer. *J. Exp. Med.* **161** (5), 1135–1151.

D'Urso, C. M., Wang, Z. G., Cao, Y., Tatake, R., Zeff, R. A., and Ferrone, S. (1991). Lack of HLA class I antigen expression by cultured melanoma cells FO-1 due to a defect in B2m gene expression. *J. Clin. Invest.* **87**(1), 284–292.

Ercolini, A. M., Ladle, B. H., Manning, E. A., Pfannenstiel, L. W., Armstrong, T. D., Machiels, J. P., Bieler, J. G., Emens, L. A., Reilly, R. T., and Jaffee, E. M. (2005). Recruitment of latent pools of high-avidity CD8(+) T cells to the antitumor immune response. *J. Exp. Med.* **201**(10), 1591–1602.

Esteban, F., Concha, A., Huelin, C., Perez-Ayala, M., Pedrinaci, S., Ruiz-Cabello, F., and Garrido, F. (1989). Histocompatibility antigens in primary and metastatic squamous cell carcinoma of the larynx. *Int. J. Cancer* **43**(3), 436–442.

Esteban, F., Concha, A., Delgado, M., Perez-Ayala, M., Ruiz-Cabello, F., and Garrido, F. (1990). Lack of MHC class I antigens and tumour aggressiveness of the squamous cell carcinoma of the larynx. *Br. J. Cancer* **62**(6), 1047–1051.

Esteban, F., Redondo, M., Delgado, M., Garrido, F., and Ruiz-Cabello, F. (1996). MHC class I antigens and tumour-infiltrating leucocytes in laryngeal cancer: Long-term follow-up. *Br. J. Cancer* **74**(11), 1801–1804.

Fearon, E. R., and Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell* **61**(5), 759–767.

Ferrone, S., and Marincola, F. M. (1995). Loss of HLA class I antigens by melanoma cells: Molecular mechanisms, functional significance and clinical relevance. *Immunol. Today* **16**(10), 487–494.

Finn, O. J. (2003). Cancer vaccines: Between the idea and the reality. *Nat. Rev. Immunol.* **3**(8), 630–641.

Gorelik, L., and Flavell, R. A. (2000). Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* **12**(2), 171–181.

Greten, F. R., Eckmann, L., Greten, T. F., Park, J. M., Li, Z. W., Egan, L. J., Kagnoff, M. F., and Karin, M. (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118**(3), 285–296.

Halak, B. K., Maguire, H. C., Jr., and Lattime, E. C. (1999). Tumor-induced interleukin-10 inhibits type 1 immune responses directed at a tumor antigen as well as a non-tumor antigen present at the tumor site. *Cancer Res.* **59,** 911–917.

Hasegawa, Y., Takanashi, S., Kanehira, Y., Tsushima, T., Imai, T., and Okumura, K. (2001). Transforming growth factor-beta1 level correlates with angiogenesis, tumor progression, and prognosis in patients with nonsmall cell lung carcinoma. *Cancer* **91**(5), 964–971.

Hatachi, S., Iwai, Y., Kawano, S., Morinobu, S., Kobayashi, M., Koshiba, M., Saura, R., Kurosaka, M., Honjo, T., and Kumagai, S. (2003). CD4+ PD-1+ T cells accumulate as unique anergic cells in rheumatoid arthritis synovial fluid. *J. Rheumatol.* **30**(7), 1410–1419.

Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J. V., Steinman, R. M., and Nussenzweig, M. C. (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* **194**(6), 769–779.

Haywood, G. R., and McKhann, C. F. (1971). Antigenic specificities on murine sarcoma cells. Reciprocal relationship between normal transplantation antigens (H-2) and tumor-specific immunogenicity. *J. Exp. Med.* **133**(6), 1171–1187.

Hilders, C. G., Houbiers, J. G., Krul, E. J., and Fleuren, G. J. (1994). The expression of histocompatibility-related leukocyte antigens in the pathway to cervical carcinoma. *Am. J. Clin. Pathol.* **101**(1), 5–12.

Hirano, F., Kaneko, K., Tamura, H., Dong, H., Wang, S., Ichikawa, M., Rietz, C., Flies, D. B., Lau, J. S., Zhu, G., Tamada, K., and Chen, L. (2005). Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res.* **65**(3), 1089–1096.

Huang, A. Y., Golumbek, P., Ahmadzadeh, M., Jaffee, E., Pardoll, D., and Levitsky, H. (1994). Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* **264** (5161), 961–965.

Huang, C. T., Workman, C. J., Flies, D., Pan, X., Marson, A. L., Zhou, G., Hipkiss, E. L., Ravi, S., Kowalski, J., Levitsky, H. I., Powell, J. D., Pardoll, D. M., *et al.* (2004). Role of LAG-3 in regulatory T cells. *Immunity* **21**(4), 503–513.

Hugo, P., Boyd, R. L., Waanders, G. A., Petrie, H. T., and Scollay, R. (1991). Timing of deletion of autoreactive V beta 6+ cells and down-modulation of either CD4 or CD8 on phenotypically distinct CD4+8+ subsets of thymocytes expressing intermediate or high levels of T cell receptor. *Int. Immunol.* **3**(3), 265–272.

Hui, K., Grosveld, F., and Festenstein, H. (1984). Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation. *Nature* **311**(5988), 750–752.

Hurchla, M. A., Sedy, J. R., Gavrieli, M., Drake, C. G., Murphy, T. L., and Murphy, K. M. (2005). B and T lymphocyte attenuator exhibits structural and expression polymorphisms and is highly induced in anergic CD4+ T cells. *J. Immunol.* **174**(6), 3377–3385.

Ishida, Y., Agata, Y., Shibahara, K., and Honjo, T. (1992). Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* **11** (11), 3887–3895.

Jager, E., Ringhoffer, M., Altmannsberger, M., Arand, M., Karbach, J., Jager, D., Oesch, F., and Knuth, A. (1997). Immunoselection *in vivo*: Independent loss of MHC class I and melanocyte differentiation antigen expression in metastatic melanoma. *Int. J. Cancer* **71**(2), 142–147.

Jones, P. A., and Baylin, S. B. (2002). The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* **3**(6), 415–428.

Kappler, J. W., Roehm, N., and Marrack, P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell* **49**(2), 273–280.

Karin, M., and Greten, F. R. (2005). NF-kappaB: Linking inflammation and immunity to cancer development and progression. *Nat. Rev. Immunol.* **5**(10), 749–759.

Karre, K., Ljunggren, H. G., Piontek, G., and Kiessling, R. (1986). Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* **319**(6055), 675–678.

Klein, S. C., Ljunggren, H. G., Piontek, G., and Kiessling, R. (1995). IL6 and IL6 receptor expression in Burkitt's lymphoma and lymphoblastoid cell lines: Promotion of IL6 receptor expression by EBV. *Hematol. Oncol.* **13**(3), 121–130.

Koopman, L. A., Corver, W. E., van der Slik, A. R., Giphart, M. J., and Fleuren, G. J. (2000). Multiple genetic alterations cause frequent and heterogeneous human histocompatibility leukocyte antigen class I loss in cervical cancer. *J. Exp. Med.* **191**(6), 961–976.

Kortylewski, M., Kujawski, M., Wang, T., Wei, S., Zhang, S., Pilon-Thomas, S., Niu, G., Kay, H., Mule, J., Kerr, W. G., Jove, R., Pardoll, D., *et al.* (2005). Inhibiting *Stat3* signalling in the hematopoietic system elicits multicomponent antitumor immunity. *Nature Med.* **11,** 1314–1321.

Kusmartsev, S., and Gabrilovich, D. I. (2006). Role of immature myeloid cells in mechanisms of immune evasion in cancer. *Cancer Immunol. Immunother.* **55,** 1–9.

Kusmartsev, S., Nefedova, Y., Yoder, D., and Gabrilovich, D. I. (2004). Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J. Immunol.* **172**(2), 989–999.

Lanier, L. L., and Phillips, J. H. (1996). Inhibitory MHC class I receptors on NK cells and T cells. *Immunol. Today* **17**(2), 86–91.

Laping, N. J., Grygielko, E., Mathur, A., Butter, S., Bomberger, J., Tweed, C., Martin, W., Fornwald, J., Lehr, R., Harling, J., Gaster, L., Callahan, J. F., *et al.* (2002). Inhibition of transforming growth factor (TGF)-beta1-induced extracellular matrix with a novel inhibitor of the TGF-beta type I receptor kinase activity: SB-431542. *Mol. Pharmacol.* **62**(1), 58–64.

Lauritzsen, G. F., Hofgaard, P. O., Schenck, K., and Bogen, B. (1998). Clonal deletion of thymocytes as a tumor escape mechanism. *Int. J. Cancer* **78**(2), 216–222.

Lechner, O., Lauber, J., Franzke, A., Sarukhan, A., von Boehmer, H., and Buer, J. (2001). Fingerprints of anergic T cells. *Curr. Biol.* **11**(8), 587–595.

Lethe, B., van den, E. B., Van Pel, A., Corradin, G., and Boon, T. (1992). Mouse tumor rejection antigens P815A and P815B: Two epitopes carried by a single peptide. *Eur. J. Immunol.* **22**(9), 2283–2288.

Letterio, J. J., and Roberts, A. B. (1998). Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol.* **16,** 137–161.

Lin, E. Y., Gouon-Evans, V., Nguyen, A. V., and Pollard, J. W. (2002). The macrophage growth factor CSF-1 in mammary gland development and tumor progression. *J. Mammary Gland Biol. Neoplasia* **7**(2), 147–162.

Lind, M. H., Rozell, B., Wallin, R. P., van Hogerlinden, M., Ljunggren, H. G., Toftgard, R., and Sur, I. (2004). Tumor necrosis factor receptor 1-mediated signaling is required for skin cancer development induced by NF-kappaB inhibition. *Proc. Natl. Acad. Sci. USA* **101**(14), 4972–4977.

Liyanage, U. K., Moore, T. T., Joo, H. G., Tanaka, Y., Herrmann, V., Doherty, G., Drebin, J. A., Strasberg, S. M., Eberlein, T. J., Goedegebuure, P. S., and Linehan, D. C. (2002). Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J. Immunol.* **169**(5), 2756–2761.

Ljunggren, H. G., and Karre, K. (1985). Host resistance directed selectively against H-2-deficient lymphoma variants. Analysis of the mechanism. *J. Exp. Med.* **162**(6), 1745–1759.

Lu, X., and Lane, D. P. (1993). Differential induction of transcriptionally active p53 following UV or ionizing radiation: Defects in chromosome instability syndromes? *Cell* **75**(4), 765–778.

Lucas, C., Bald, L. N., Fendly, B. M., Mora-Worms, M., Figari, I. S., Patzer, E. J., and Palladino, M. A. (1990). The autocrine production of transforming growth factor-beta 1 during lymphocyte activation. A study with a monoclonal antibody-based ELISA. *J. Immunol.* **145**(5), 1415–1422.

Lyman, M. A., Aung, S., Biggs, J. A., and Sherman, L. A. (2004). A spontaneously arising pancreatic tumor does not promote the differentiation of naive CD8+ T lymphocytes into effector CTL. *J. Immunol.* **172**(11), 6558–6567.

MacDonald, H. R., Schneider, R., Lees, R. K., Howe, R. C., Acha-Orbea, H., Festenstein, H., Zinkernagel, R. M., and Hengartner, H. (1988). T-cell receptor V beta use predicts reactivity and tolerance to Mlsa-encoded antigens. *Nature* **332**(6159), 40–45.

Machiels, J. P., Reilly, R. T., Emens, L. A., Ercolini, A. M., Lei, R. Y., Weintraub, D., Okoye, F. I., and Jaffee, E. M. (2001). Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. *Cancer Res.* **61**(9), 3689–3697.

Marincola, F. M., Jaffee, E. M., Hicklin, D. J., and Ferrone, S. (2000). Escape of human solid tumors from T-cell recognition: Molecular mechanisms and functional significance. *Adv. Immunol.* **74,** 181–273.

Mazzoni, A., Bronte, V., Visintin, A., Spitzer, J. H., Apolloni, E., Serafini, P., Zanovello, P., and Segal, D. M. (2002). Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. *J. Immunol.* **168**(2), 689–695.

McHugh, R. S., Whitters, M. J., Piccirillo, C. A., Young, D. A., Shevach, E. M., Collins, M., and Byrne, M. C. (2002). CD4(+)CD25(+) immunoregulatory T cells: Gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* **16**(2), 311–323.

Medema, J. P., Schuurhuis, D. H., Rea, D., van Tongeren, J., de Jong, J., Bres, S. A., Laban, S., Toes, R. E., Toebes, M., Schumacher, T. N., Bladergroen, B. A., Ossendorp, F., *et al.* (2001). Expression of the serpin serine protease inhibitor 6 protects dendritic cells from cytotoxic T lymphocyte-induced apoptosis: Differential modulation by T helper type 1 and type 2 cells. *J. Exp. Med.* **194**(5), 657–667.

Mellor, A. L., Chandler, P., Baban, B., Hansen, A. M., Marshall, B., Pihkala, J., Waldmann, H., Cobbold, S., Adams, E., and Munn, D. H. (2004). Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int. Immunol.* **16**(10), 1391–1401.

Mizoguchi, H., O'Shea, J. J., Longo, D. L., Loeffler, C. M., McVicar, D. W., and Ochoa, A. C. (1992). Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. *Science* **258**(5089), 1795–1798.

Moretta, A., Biassoni, R., Bottino, C., Pende, D., Vitale, M., Poggi, A., Mingari, M. C., and Moretta, L. (1997). Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol. Rev.* **155,** 105–117.

Munn, D. H., Sharma, M. D., Lee, J. R., Jhaver, K. G., Johnson, T. S., Keskin, D. B., Marshall, B., Chandler, P., Antonia, S. J., Burgess, R., Slingluff, C. L., Jr., and Mellor, A. L. (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* **297**(5588), 1867–1870.

Munn, D. H., Sharma, M. D., Hou, D., Baban, B., Lee, J. R., Antonia, S. J., Messina, J. L., Chandler, P., Koni, P. A., and Mellor, A. L. (2004). Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J. Clin. Invest.* **114**(2), 280–290.

Munn, D. H., Sharma, M. D., Baban, B., Harding, H. P., Zhang, Y., Ron, D., and Mellor, A. L. (2005). GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* **22**(5), 633–642.

Natali, P., Bigotti, A., Cavaliere, R., Liao, S. K., Taniguchi, M., Matsui, M., and Ferrone, S. (1985). Heterogeneous expression of melanoma-associated antigens and HLA antigens by primary and multiple metastatic lesions removed from patients with melanoma. *Cancer Res.* **45**(6), 2883–2889.

Natali, P. G., Viora, M., Nicotra, M. R., Giacomini, P., Bigotti, A., and Ferrone, S. (1983). Antigenic heterogeneity of skin tumors of nonmelanocyte origin: Analysis with monoclonal antibodies to tumor-associated antigens and to histocompatibility antigens. *J. Natl. Cancer Inst.* **71,** 439–447.

Natali, P. G., Bigotti, A., Nicotra, M. R., Viora, M., Manfredi, D., and Ferrone, S. (1984). Distribution of human Class I (HLA-A,B,C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. *Cancer Res.* **44**(10), 4679–4687.

Natali, P. G., Nicotra, M. R., Bigotti, A., Venturo, I., Marcenaro, L., Giacomini, P., and Russo, C. (1989). Selective changes in expression of HLA class I polymorphic determinants in human solid tumors. *Proc. Natl. Acad. Sci. USA* **86**(17), 6719–6723.

Nefedova, Y., Huang, M., Kusmartsev, S., Bhattacharya, R., Cheng, P., Salup, R., Jove, R., and Gabrilovich, D. (2004). Hyperactivation of *Stat3* is involved in abnormal differentiation of dendritic cells in cancer. *J. Immunol.* **172**(1), 464–474.

Nefedova, Y., Nagaraj, S., Rosenbauer, A., Muro-Cacho, C., Sebti, S. M., and Gabrilovich, D. I. (2005). Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res.* **65**(20), 9525–9535.

Nguyen, L. T., Radhakrishnan, S., Ciric, B., Tamada, K., Shin, T., Pardoll, D. M., Chen, L., Rodriguez, M., and Pease, L. R. (2002a). Cross-linking the B7 family molecule B7-DC directly activates immune functions of dendritic cells. *J. Exp. Med.* **196**(10), 1393–1398.

Nguyen, L. T., Elford, A. R., Murakami, K., Garza, K. M., Schoenberger, S. P., Odermatt, B., Speiser, D. E., and Ohashi, P. S. (2002b). Tumor growth enhances cross-presentation leading to limited T cell activation without tolerance. *J. Exp. Med.* **195**(4), 423–435.

Nigam, A., Yacavone, R. F., Zahurak, M. L., Johns, C. M. S., Pardoll, D. M., Piantadosi, S., Levitsky, H. I., and Nelson, W. G. (1998). Immunomodulatory properties of antineoplastic drugs administered in conjunction with GM-CSF-secreting cancer cell vaccines. *Int. J. Oncol.* **12**(1), 161–170.

Nishimura, H., Nose, M., Hiai, H., Minato, N., and Honjo, T. (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11**(2), 141–151.

Nishimura, H., Okazaki, T., Tanaka, Y., Nakatani, K., Hara, M., Matsumori, A., Sasayama, S., Mizoguchi, A., Hiai, H., Minato, N., and Honjo, T. (2001). Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* **291**(5502), 319–322.

Niu, G., Wright, K. L., Huang, M., Song, L., Haura, E., Turkson, J., Zhang, S., Wang, T., Sinibaldi, D., Coppola, D., Heller, R., Ellis, L. M., *et al.* (2002). Constitutive *Stat3* activity up-regulates VEGF expression and tumor angiogenesis. *Oncogene* **21**(13), 2000–2008.

North, R. J. (1982). Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J. Exp. Med.* **155**(4), 1063–1074.

Ochsenbein, A. F. (2005). Immunological ignorance of solid tumors. *Springer Semin Immunopathol.* **27,** 19–35.

Ochsenbein, A. F., Klenerman, P., Karrer, U., Ludewig, B., Pericin, M., Hengartner, H., and Zinkernagel, R. M. (1999). Immune surveillance against a solid tumor fails because of immunological ignorance. *Proc. Natl. Acad. Sci. USA* **96**(5), 2233–2238.

Ohnmacht, G. A., Wang, E., Mocellin, S., Abati, A., Filie, A., Fetsch, P., Riker, A. I., Kammula, U. S., Rosenberg, S. A., and Marincola, F. M. (2001). Short-term kinetics of tumor antigen expression in response to vaccination. *J. Immunol.* **167**(3), 1809–1820.

Pardoll, D., and Allison, J. (2004). Cancer immunotherapy: Breaking the barriers to harvest the crop. *Nat. Med.* **10**(9), 887–892.

Pepper, M. S. (1997). Transforming growth factor-beta: Vasculogenesis, angiogenesis, and vessel wall integrity. *Cytokine Growth Factor Rev.* **8**(1), 21–43.

Perez, M., Cabrera, T., Lopez Nevot, M. A., Gomez, M., Peran, F., Ruiz-Cabello, F., and Garrido, F. (1986). Heterogeneity of the expression of class I and II HLA antigens in human breast carcinoma. *J. Immunogenet.* **13**(2–3), 247–253.

Phan, G. Q., Yang, J. C., Sherry, R. M., Hwu, P., Topalian, S. L., Schwartzentruber, D. J., Restifo, N. P., Haworth, L. R., Seipp, C. A., Freezer, L. J., Morton, K. E., Mavroukakis, S. A., *et al.* (2003). Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl. Acad. Sci. USA* **100**(14), 8372–8377.

Quill, H., and Schwartz, R. H. (1987). Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: Specific induction of a long-lived state of proliferative nonresponsiveness. *J. Immunol.* **138**(11), 3704–3712.

Ramsdell, F., and Fowlkes, B. J. (1992). Maintenance of *in vivo* tolerance by persistence of antigen. *Science* **257**(5073), 1130–1134.

Restifo, N. P., Marincola, F. M., Kawakami, Y., Taubenberger, J., Yannelli, J. R., and Rosenberg, S. A. (1996). Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J. Natl. Cancer Inst.* **88**(2), 100–108.

Riker, A., Cormier, J., Panelli, M., Kammula, U., Wang, E., Abati, A., Fetsch, P., Lee, K. H., Steinberg, S., Rosenberg, S., and Marincola, F. (1999). Immune selection after antigen-specific immunotherapy of melanoma. *Surgery* **126**(2), 112–120.

Rowe, M., Khanna, R., Jacob, C. A., Argaet, V., Kelly, A., Powis, S., Belich, M., Croom-Carter, D., Lee, S., Burrows, S. R., Trowsdale, J., Moss, D. J., *et al.* (1995). Restoration of endogenous antigen processing in Burkitt's lymphoma cells by Epstein-Barr virus latent membrane protein-1: Coordinate up-regulation of peptide transporters and HLA-class I antigen expression. *Eur. J. Immunol.* **25**(5), 1374–1384.

Ruiz-Cabello, F., Lopez Nevot, M. A., Gutierrez, J., Oliva, M. R., Romero, C., Ferron, A., Esteban, F., Huelin, C., Piris, M. A., and Rivas, C. (1989). Phenotypic expression of histocompatibility antigens in human primary tumours and metastases. *Clin. Exp. Metastasis* **7**(2), 213–226.

Ruiz-Cabello, F., Perez-Ayala, M., Gomez, O., Redondo, M., Concha, A., Cabrera, T., and Garrido, F. (1991). Molecular analysis of MHC-class-I alterations in human tumor cell lines. *Int. J. Cancer Suppl.* **6,** 123–130.

Saito, H., Tsujitani, S., Oka, S., Kondo, A., Ikeguchi, M., Maeta, M., and Kaibara, N. (1999). The expression of transforming growth factor-beta1 is significantly correlated with the expression of vascular endothelial growth factor and poor prognosis of patients with advanced gastric carcinoma. *Cancer* **86**(8), 1455–1462.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**(3), 1151–1164.

Salama, A. D., Chitnis, T., Imitola, J., Ansari, M. J., Akiba, H., Tushima, F., Azuma, M., Yagita, H., Sayegh, M. H., and Khoury, S. J. (2003). Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. *J. Exp. Med.* **198**(1), 71–78.

Sanda, M. G., Restifo, N. P., Walsh, J. C., Kawakami, Y., Nelson, W. G., Pardoll, D. M., and Simons, J. W. (1995). Molecular characterization of defective antigen processing in human prostate cancer. *J. Natl. Cancer Inst.* **87**(4), 280–285.

Sano, S., Itami, S., Takeda, K., Tarutani, M., Yamaguchi, Y., Miura, H., Yoshikawa, K., Akira, S., and Takeda, J. (1999). Keratinocyte-specific ablation of *Stat3* exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J.* **18**(17), 4657–4668.

Scheibenbogen, C., Weyers, I., Ruiter, D., Willhauck, M., Bittinger, A., and Keilholz, U. (1996). Expression of gp100 in melanoma metastases resected before or after treatment with IFN alpha and IL-2. *J. Immunother. Emphasis Tumor Immunol.* **19**(5), 375–380.

Schmid, P., Itin, P., and Rufli, T. (1995). *In situ* analysis of transforming growth factor-beta s (TGF-beta 1, TGF-beta 2, TGF-beta 3), and TGF-beta type II receptor expression in malignant melanoma. *Carcinogenesis* **16**(7), 1499–1503.

Schmielau, J., and Finn, O. J. (2001). Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Cancer Res.* **61**(12), 4756–4760.

Schwartz, R. H. (1992). Costimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* **71**(7), 1065–1068.

Schwartz, R. H. (1999). Immunological tolerance. *In* "Fundamental Immunology" (W. E. Paul, Ed.), pp. 701–740. Lippincott-Raven, Philadelphia.

Schwartz, R. H., *et al.* (1989). T-cell clonal anergy. *Cold Spring Harb. Symp. Quant. Biol.* **54**(Pt. 2), 605–610.

Sedy, J. R., Gavrieli, M., Potter, K. G., Hurchla, M. A., Lindsley, R. C., Hildner, K., Scheu, S., Pfeffer, K., Ware, C. F., Murphy, T. L., and Murphy, K. M. (2005). B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat. Immunol.* **6**(1), 90–98.

Seliger, B., Hohne, A., Knuth, A., Bernhard, H., Ehring, B., Tampe, R., and Huber, C. (1996). Reduced membrane major histocompatibility complex class I density and stability in a subset of human renal cell carcinomas with low TAP and LMP expression. *Clin. Cancer Res.* **2**(8), 1427–1433.

Serafini, P., Carbley, R., Noonan, K. A., Tan, G., Bronte, V., and Borrello, I. (2004). High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res.* **64**(17), 6337–6343.

Shariat, S. F., Kim, J. H., Andrews, B., Kattan, M. W., Wheeler, T. M., Kim, I. Y., Lerner, S. P., and Slawin, K. M. (2001). Preoperative plasma levels of transforming growth factor beta(1) strongly predict clinical outcome in patients with bladder carcinoma. *Cancer* **92**(12), 2985–2992.

Shariat, S. F., Shalev, M., Menesses-Diaz, A., Kim, I. Y., Kattan, M. W., Wheeler, T. M., and Slawin, K. M. (2001). Preoperative plasma levels of transforming growth factor beta(1) (TGF-beta(1) strongly predict progression in patients undergoing radical prostatectomy. *J. Clin. Oncol.* **19**(11), 2856–2864.

Shevach, E. M. (2004). Regulatory/suppressor T cells in health and disease. *Arthritis Rheum.* **50**(9), 2721–2724.

Shin, T., Kennedy, G., Gorski, K., Tsuchiya, H., Koseki, H., Azuma, M., Yagita, H., Chen, L., Powell, J., Pardoll, D., and Housseau, F. (2003). Cooperative B7-1/2 (CD80/CD86) and B7-DC costimulation of CD4+ T cells independent of the PD-1 receptor. *J. Exp. Med.* **198**(1), 31–38.

Staveley-O'Carroll, K., Sotomayor, E., Montgomery, J., Borrello, I., Hwang, L., Fein, S., Pardoll, D., and Levitsky, H. (1998). Induction of antigen-specific T cell anergy: An early event in the course of tumor progression. *Proc. Natl. Acad. Sci. USA* **95**(3), 1178–1183.

Steinman, R. M., Hawiger, D., and Nussenzweig, M. C. (2003). Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **21,** 685–711.

Sumimoto, H., Hirata, K., Yamagata, S., Miyoshi, M., Miyagishi, M., Taira, K., and Kawakami, Y. (2006). Effective inhibition of cell growth and invasion of melanoma by combined suppression of BRAF (V599E) and Skp2 with lentiviral RNAi. *Int. J. Cancer* **118,** 472–476.

Surh, C. D., and Sprent, J. (1994). T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature* **372**(6501), 100–103.

Terabe, M., and Berzofsky, J. A. (2004). Immunoregulatory T cells in tumor immunity. *Curr. Opin. Immunol.* **16**(2), 157–162.

Thompson, R. H., Gillett, M. D., Cheville, J. C., Lohse, C. M., Dong, H., Webster, W. S., Krejci, K. G., Lobo, J. R., Sengupta, S., Chen, L., Zincke, H., Blute, M. L., *et al.* (2004). Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. *Proc. Natl. Acad. Sci. USA* **101**(49), 17174–17179.

Tseng, S. Y., Otsuji, M., Gorski, K., Huang, X., Slansky, J. E., Pai, S. I., Shalabi, A., Shin, T., Pardoll, D. M., and Tsuchiya, H. (2001). B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J. Exp. Med.* **193**(7), 839–846.

Turkson, J., Bowman, T., Garcia, R., Caldenhoven, E., De Groot, R. P., and Jove, R. (1998). *Stat3* activation by Src induces specific gene regulation and is required for cell transformation. *Mol. Cell. Biol.* **18**(5), 2545–2552.

Turkson, J., Zhang, S., Mora, L. B., Burns, A., Sebti, S., and Jove, R. (2005). A novel platinum compound inhibits constitutive *Stat3* signaling and induces cell cycle arrest and apoptosis of malignant cells. *J. Biol. Chem.* **280**(38), 32979–32988.

Urban, J. L., Burton, R. C., Holland, J. M., Kripke, M. L., and Schreiber, H. (1982). Mechanisms of syngeneic tumor rejection. Susceptibility of host-selected progressor variants to various immunological effector cells. *J. Exp. Med.* **155**(2), 557–573.

Urban, J. L., Kripke, M. L., and Schreiber, H. (1986). Stepwise immunologic selection of antigenic variants during tumor growth. *J. Immunol.* **137**(9), 3036–3041.

Uyttenhove, C., Maryanski, J., and Boon, T. (1983). Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression. *J. Exp. Med.* **157**(3), 1040–1052.

van den Ingh, H. F., Ruiter, D. J., Griffioen, G., van Muijen, G. N., and Ferrone, S. (1987). HLA antigens in colorectal tumours–low expression of HLA class I antigens in mucinous colorectal carcinomas. *Br. J. Cancer* **55**(2), 125–130.

van Driel, W. J., Tjiong, M. Y., Hilders, C. G., Trimbos, B. J., and Fleuren, G. J. (1996). Association of allele-specific HLA expression and histopathologic progression of cervical carcinoma. *Gynecol. Oncol.* **62**(1), 33–41.

Vicari, A. P., Caux, C., and Trinchieri, G. (2002). Tumour escape from immune surveillance through dendritic cell inactivation. *Semin. Cancer Biol.* **12**(1), 33–42.

von Boehmer, H. (2005). Mechanisms of suppression by suppressor T cells. *Nat. Immunol.* **6**(4), 338–344.

Wallich, R., Bulbuc, N., Hammerling, G. J., Katzav, S., Segal, S., and Feldman, M. (1985). Abrogation of metastatic properties of tumour cells by *de novo* expression of H-2K antigens following H-2 gene transfection. *Nature* **315**(6017), 301–305.

Wang, T., Niu, G., Kortylewski, M., Burdelya, L., Shain, K., Zhang, S., Bhattacharya, R., Gabrilovich, D., Heller, R., Coppola, D., Dalton, W., Jove, R., *et al.* (2004). Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat. Med.* **10**(1), 48–54.

Wang, Z., Cao, Y., Albino, A. P., Zeff, R. A., Houghton, A., and Ferrone, S. (1993). Lack of HLA class I antigen expression by melanoma cells SK-MEL-33 caused by a reading frameshift in beta 2-microglobulin messenger RNA. *J. Clin. Invest.* **91**(2), 684–692.

Ward, P. L., Koeppen, H., Hurteau, T., and Schreiber, H. (1989). Tumor antigens defined by cloned immunological probes are highly polymorphic and are not detected on autologous normal cells. *J. Exp. Med.* **170**(1), 217–232.

Watanabe, N., Gavrieli, M., Sedy, J. R., Yang, J., Fallarino, F., Loftin, S. K., Hurchla, M. A., Zimmerman, N., Sim, J., Zang, X., Murphy, T. L., Russell, J. H., *et al.* (2003). BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nat. Immunol.* **4**(7), 670–679.

Waterhouse, P., Penninger, J. M., Timms, E., Wakeham, A., Shahinian, A., Lee, K. P., Thompson, C. B., Griesser, H., and Mak, T. W. (1995). Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* **270**(5238), 985–988.

Whitwell, H. L., Hughes, H. P., Moore, M., and Ahmed, A. (1984). Expression of major histocompatibility antigens and leucocyte infiltration in benign and malignant human breast disease. *Br. J. Cancer* **49**(2), 161–172.

Wortzel, R. D., Philipps, C., and Schreiber, H. (1983). Multiple tumour-specific antigens expressed on a single tumour cell. *Nature* **304**(5922), 165–167.

Yee, C., Thompson, J. A., Byrd, D., Riddell, S. R., Roche, P., Celis, E., and Greenberg, P. D. (2002). Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: *In vivo* persistence, migration, and antitumor effect of transferred T cells. *Proc. Natl. Acad. Sci. USA* **99**(25), 16168–16173.

Young, M. R., Petruzzelli, G. J., Kolesiak, K., Achille, N., Lathers, D. M., and Gabrilovich, D. I. (2001). Human squamous cell carcinomas of the head and neck chemoattract immune suppressive CD34(+) progenitor cells. *Hum. Immunol.* **62**(4), 332–341.

Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J., Suresh, M., Altman, J. D., and Ahmed, R. (1998). Viral immune evasion due to persistence of activated T cells without effector function. *J. Exp. Med.* **188**(12), 2205–2213.

Zea, A. H., Rodriguez, P. C., Atkins, M. B., Hernandez, C., Signoretti, S., Zabaleta, J., McDermott, D., Quiceno, D., Youmans, A., O'Neill, A., Mier, J., and Ochoa, A. C. (2005). Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: A mechanism of tumor evasion. *Cancer Res.* **65**(8), 3044–3048.

Zhang, Q., Yang, X., Pins, M., Javonovic, B., Kuzel, T., Kim, S. J., Parijs, L. V., Greenberg, N. M., Liu, V., Guo, Y., and Lee, C. (2005). Adoptive transfer of tumor-reactive transforming growth factor-beta-insensitive CD8+ T cells: Eradication of autologous mouse prostate cancer. *Cancer Res.* **65**(5), 1761–1769.

Zuk, J. A., and Walker, R. A. (1987). Immunohistochemical analysis of HLA antigens and mononuclear infiltrates of benign and malignant breast. *J. Pathol.* **152,** 275–275.