



Review

Immune suppression in cancer: Effects on immune cells, mechanisms and future therapeutic intervention

Theresa L. Whiteside*

University of Pittsburgh Cancer Institute, Department of Pathology, School of Medicine, Hillman Cancer Center, 5117 Centre Avenue, Suite 1.27, Pittsburgh, PA 15213, USA

Abstract

Evidence indicates that the healthy immune system is necessary for control of malignant disease and that immune suppression associated with cancer contributes to its progression. Tumors have developed strategies to successfully evade the host immune system, and various molecular and cellular mechanisms responsible for tumor evasion have been identified. Certain of these mechanisms target immune anti-tumor effector cells. Dysfunction and apoptosis of these cells in the tumor-bearing host creates an immune imbalance that cannot be corrected by immunotherapies aimed only at activation of anti-tumor immune responses. Reversal of existing immune dysfunction(s) and normalization of lymphocyte homeostasis in patients with cancer needs to be a part of future cancer immunotherapy. Therapeutic strategies are being designed to correct the immune imbalance, deliver adequate in vivo stimulation, transfer effector T cells capable of in vivo expansion and provide protection for the immune effector cells re-populating the host. Survival of these cells and long-term memory development in patients with malignancy are necessary for improving clinical benefits of cancer immunotherapies.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Cancer; Immune dysfunction; Lymphocyte apoptosis; Tumor escape; Immune therapies

Contents

1. Host immune competence and cancer	00
2. Is tumor progression helped by immune cells?	00
3. Inflammation and cancer	00
4. How tumors evade the host immune system	00
5. Mechanisms of tumor evasion	00
6. Reversal of immune dysfunction as a goal of cancer immunotherapy	00
7. Conclusions and future prospects	00
References	00

1. Host immune competence and cancer

The involvement of the host immune system in control of cancer progression has been suspected but remained inconclusive for many years. This is because of the lack of convincing evidence for a direct link between cancer development and lower immune competence in individuals who succumb

to cancer. However, standard tests for measuring immune competence to tumor-associated antigens (TAA), similar to those available for the assessment of responses to bacterial, viral or fungal antigens, have not been available. Also, TAA are largely self-antigens and, therefore, TAA-specific immune responses, whether cellular or humoral, are weak and difficult to measure. Innate immunity, which according to the immune surveillance theory is responsible for early detection and elimination of malignant cells [1,2], may be inefficient in patients who develop malignancy. Evidence is

* Tel.: +1 412 624 0096; fax: +1 412 624 0264.
E-mail address: whitesidetl@upmc.edu.

convincing that individuals who are older, who have been on immuno-suppressive medications over prolonged periods of time or have underlying immune abnormalities, such as an autoimmune disease or a chronic infection (e.g., AIDS) are particularly at risk of malignancy [3,4]. Aside from genetic predisposition to cancer [5] or previous viral infections, such as hepatitis, Epstein Barr virus, or herpes virus infections or HIV, all of which are associated with the development of specific cancer types [3,4], most common risk factors for cancer are age, poor nutrition, stress, smoking and excessive alcohol consumption [6,7]. All of the above are also associated with more or less pronounced abnormalities in the immune system [8].

Testing for immune competence in populations at high risk for malignancy has not been routinely performed. There are indications, however, that a loss of immune competence may be an important risk factor. For example, low natural killer (NK) activity has been reported in familial breast cancer patients as well as their clinically asymptomatic first degree relatives [9]. Other studies support the conclusion that members of cancer families have lower levels of natural cytotoxic activity than age-matched individuals without cancer in first degree relatives [10,11]. These studies suggest that among the unaffected family members, persons with lower NK cell activity may be at higher risk of cancer [11]. In combination with evidence for significantly depressed levels of NK cell activity reported for cancer patients with advanced disease, these studies implicate persistently low NK cell activity as a risk for developing malignancy.

Also, delayed type hypersensitivity responses (DTH) to recall antigens were found to be absent in individuals who later developed a malignancy [12]. However, these are sporadic or anecdotal reports that have not been uniformly accepted as evidence for a lack of immune surveillance in individuals at high risk of cancer. Such evidence is better sought in animal models of tumor growth, where immunization with a relevant tumor epitopes slows or completely inhibits tumor progression and prolongs survival [13]. Also, animals that are deficient in immune cell subsets or genetically altered to eliminate molecular signals necessary for immune responses have been shown to be highly susceptible to cancer development [14]. This type of evidence is taken to mean that the host immune competence in respect to innate as well as adaptive immunity is important and perhaps necessary for cancer prevention.

A more convincing argument can be made for the role of the immune system in control of tumor progression than its prevention. Both in animal models of tumor growth and in humans with cancer, it is clear that the host usually makes an immune response to the tumor. Tumor-specific cytolytic T lymphocytes (CTL) and IgG antibodies specific for tumor epitopes are detectable in cancer-bearing hosts, using sensitive modern technologies. In particular, the tetramer technology has contributed to our ability to detect and estimate the frequency of T cells specific for tumor epitopes [15]. T cells able to recognize MHC class I- or class II-restricted peptides

can now be measured in the circulation or tissues of patients with cancer using tetramers and flow cytometry [16]. For example, we have obtained quantitative estimates of wild-type sequence (wt) p53 peptide-specific T cells in HLA-A2+ patients with head and neck cancer (HNC) as well as healthy age matched controls [17]. While the frequency of T cells specific for wt p53 peptides is higher in the peripheral circulation of HNC patients than normal controls (NC), T-cell precursors with TCR able to recognize such peptides are detectable in blood of most HLA-A2+ normal donors [17]. Similar results are available for other tumor-associated peptides in patients with other cancers and NC cohorts [18,19]. Functional *ex vivo* assays, such as proliferation, cytotoxicity or cytokine production in response to tumor-associated peptides, confirm these phenotypic data, although *ex vivo* responses to these epitopes are often weak and require *in vitro* sensitization (IVS) with antigen-presenting cells (APC) and exogenous cytokines. Taken together, it is clear that T lymphocyte precursors capable of responding to self-antigens, which are often over-expressed in tumors, are detectable albeit at low frequencies in the circulation of most individuals. Such T lymphocytes are enriched in tumor tissues [20]. Antibodies to TAA are also often detectable in patients with cancer [21] and have been used as a biomarker of prognosis, as is the case with, e.g., Abs to p53 in a subgroup of HNC patients who have a particularly poor prognosis [22]. The available data are consistent with the presence of TAA-reactive precursor T cells in most individuals. However, tolerance to self prevents the generation of effective anti-tumor immune responses early on, when tumors first arise. It has been suggested that an absence of a strong “danger” signal at this time contributes to the ability of newly forming tumors to avoid recognition by the host immune system [23]. The host is immunocompetent but tolerance to self prevents generation of effective anti-tumor immunity.

2. Is tumor progression helped by immune cells?

Pre-malignant and early tumor lesions are generally well infiltrated with immune cells, largely T lymphocytes, macrophages and dendritic cells (DC), although B-cell formations resembling lymphoid follicles are sometimes present [24,25]. These immune cells, tumor-infiltrating lymphocytes (TIL), are considered to be a component of an inflammatory host response to the tumor. Over the years, considerable evidence has accumulated indicating that: (a) despite their activation phenotype, TIL are functionally compromised and (b) TIL are enriched in TAA-specific memory T cells (reviewed in [26]). Therefore, TIL accumulate in response to the tumor-initiated “signals” and, unlike other inflammatory infiltrates, contain immune cells specific for a variety of peptides expressed by tumor cells. It has been suggested that TIL elaborate cytokines and growth factors necessary for tumor growth [27], and that tumors produce chemotactic factors that actively recruit mononuclear cells,

mainly lymphocytes and macrophages in humans, to tumor sites [28]. Whether this recruitment is orchestrated by the tumor or is generated by surrounding tissue cells in response to the tumor is currently an unanswered question. It has been suggested that the recruited cells, activated in response to local “danger signals” might be a source of trophic factors for the tumor, but fail to exercise anti-tumor functions. In support of this hypothesis, recent data indicate that TIL are capable of producing cytokines in response to TAA, although their anti-tumor effector functions are either weak or absent.

In pre-malignant lesions such as dysplasias or carcinomas in situ (CIS), cells forming these lesions may be antigenically indistinguishable from normal tissue cells, although genetic abnormalities are already present [29]. It is recognized that clonally expanding tumor cells are genetically unstable and readily acquire chromosomal aberrations [29]. If these tumor cells are recognized by the host immune system, they are eliminated. This process of immune selection leads to the outgrowth of tumor cells that are genetically altered but resistant to immune detection systems. Immune selection may, in fact, promote growth of tumors resistant to immune intervention. Therefore, the role of the immune system in host protection from malignancy or in control of tumor progression remains controversial and may be dependent on characteristics of the individual tumor.

The role of immunity in tumor metastasis is also disputed. To metastasize, the tumor must possess certain unique characteristics, including the capability to penetrate the endothelium and acquire mobility within tissues as well as lymphatics or blood vessels [30]. Not surprisingly, solid tumor cells appear to be able to adopt the phenotypic characteristics of lymphoid cells that enable them to migrate using exactly the same mechanisms [31]. This phenomenon of “masquerading” provides yet another example of how tumors use the immune cells to their own advantage. At the same time, it is recognized that circulating tumor cells are particularly sensitive to lysis by natural killer cells or monocytes [32]. In the presence of anti-tumor Abs, these effector cells of innate immunity would also be able to mediate antibody-dependent cellular cytotoxicity (ADCC), thus efficiently eliminating tumor targets [33]. A tumor cell that manages to avoid such immune intervention in the peripheral blood or lymphatic circulation and arrives at a new tissue site is undoubtedly dependent on the local microenvironment for growth factors and structural support by the extracellular matrix (ECM). It has been speculated that tumor-specific immune cells responding to TAA can produce such growth factors, thus promoting metastasis formation. Again, the picture that emerges has the host immune system performing a dual role of tumor elimination or tumor promotion, perhaps depending on local circumstances and signals delivered by the tumor. More aggressive tumors might be more successful in subverting the microenvironment, including immune cells, to subserve tumor needs.

The current view of the host immune system responding to the arising or progressing tumor is conflicted by evidence

for anti-tumor effects as well as evidence for tumor-favoring trophic functions. Which of these effects prevail may be determined by the tumor and not by the host, as discussed below.

3. Inflammation and cancer

Tissue trauma normally engenders infiltration into tissue of inflammatory cells and production of the variety of cytokines or growth factors suppressing or promoting cellular proliferation. Most human tumors are infiltrated by mononuclear cells throughout various stages of their progression. Sustained inflammation at tumor sites leads to release of soluble factors and reactive oxygen species (ROS), which can contribute to generation of dysplastic changes in the genetically altered, initiated tissue cells. If inflammation becomes persistent, e.g., is driven by cell death and necrosis, the cytokine cascade that evolves could mediate either augmentation or suppression of local immune responses, depending on the cellular makeup of the microenvironment. When transformed cells, interacting with inflammatory cells and growth factors (e.g., TNF- α) at sites of chronic inflammation continue to proliferate, the persistent inflammatory process may become a crucial step in carcinogenesis. Many cancers have been associated with persistent inflammation: lung carcinomas with asbestosis or silicosis; colon cancer with inflammatory bowel disease; pancreatic cancer with pancreatitis; oral squamous cell carcinoma with gingivitis and so on [34]. If inadequately cleared bacterial or viral infections are the cause of chronic inflammation, strong “danger signals” are generated in situ, including LPS, dsRNA, CpG DNA, which engage toll-like receptors (TLR) on macrophages (M ϕ), and induce massive release of ROS, chemokines, cytokines and enzymes from M ϕ and neutrophils [35]. Transformed, genetically unstable tissue cells undergoing clonal expansion in this microenvironment have an opportunity for accumulation of additional genetic alterations. It is well known that some cancers are associated with or preceded by uncontrolled infections with pathogens and chronic inflammation, e.g., gastric cancer with *Helicobacter pylori*, cervical carcinoma with HPV, liver cancer with hepatitis B and C viruses, Kaposi’s sarcoma with HSV-8 or adult T-cell leukemia with human T-cell lymphotropic virus, and others. Recently performed studies in cytokine knock out (KO) mice have shown that pro-inflammatory cytokines, e.g., TNF- α , play a key role in inducing carcinogenesis, probably acting via the NF κ B pathway [36]. However, it should be remembered that immune surveillance, presumably with elimination of expanding transformed cells is occurring at the same time. Therefore, the tenuous balance that exists between immune surveillance and immune promotion is likely to shift, depending on the signals existing in situ as well as the host ability to modulate these signals. In this scenario, cancer progresses when the host’s immune system becomes incapable of curtailing chronic inflammation and initiating the healing process. Thus, Dr. H. Dworak’s description of the tumor as “a wound

that does not heal” is a particularly astute appraisal of this situation [37].

4. How tumors evade the host immune system

Human tumors, like viruses, have evolved an elaborate assembly of tricks designed to fool the immune system [38]. In fact, molecular mechanisms used by tumors to neutralize immune cells are “borrowed” from viruses [38]. In general, tumors employ two strategies to avoid recognition: they either “hide” from immune cells thus avoiding recognition or they proceed to disable or eliminate immune cells.

It has been recognized for a long time that tumors are adept at shedding surface antigens or down-regulating expression of key molecules necessary for interactions with immune cells (reviewed in [39]). In this way, tumors can evade the host’s immune response by being (a) poor stimulators of T cells or (b) poor targets for tumor-specific T cells (CTL). Expression of molecules such as TAA, HLA class I molecules or antigen processing machinery components (APM) is often down-regulated or altered in tumor cells [40]. As a result of abnormalities in the APM components, which might include their down-regulation, absence or mutation [41], peptides are not generated from TAA or are generated in a form not allowing for the formation of HLA class I-peptide complexes recognized by T cells [41]. Tumors are not effective antigen-presenting cells, and they frequently mis-process and mis-represent processed TAA, so that immunogenic peptides cannot be made or are defective and thus do not fit into the HLA class I groove. In this case, the trimolecular complex, HLA class I-chain- β_2m -peptide, is absent from the tumor cell surface. Alternatively, the peptide-HLA molecule complex is formed and presented but in a configuration that cannot be

recognized by CTLs. To illustrate these mechanisms, a mutation in the p53 protein, which inhibits proteasome-mediated generation of wt p53_{264–272}, abolishes the possibility for a CTL response to this normally immunogenic peptide in some patients with HNC [42]. Similarly, down-regulation or mutation in TAP1 or TAP2 prevents normal processing of TAA in HNC [41] or in melanoma cells [43]. Such examples offer a glimpse of perturbations that accumulate in tumor cells, resulting in a loss of recognition by tumor-specific T cells.

The most frequent abnormality seen in tumor cells involves changes in expression of classical HLA class I antigens [44]. As previously shown, these changes range from a total loss of HLA class I molecules to more selective down-regulation of HLA-A, B or C locus expression [44]. The frequency of HLA class I antigen loss or down-regulation has been found to range between 16 and 80% for the various tumor types analyzed by immunohistochemistry (IHC), using mAbs recognizing monomorphic HLA determinants [39]. These changes have been less evident in breast or prostate carcinomas and are most frequently observed in renal cell carcinoma (RCC) and melanoma [39]. While methods for detection of HLA class I expression in various tumors are dependent on IHC and thus may be subject to experimental bias, it is notable that down-regulation or absence of HLA class I antigen expression or APM component expression, appears to be biologically and clinically significant. Thus, the presence of these abnormalities has been related to shorter survival in patients with HNC, as recently reported [41,45].

Another aberration frequently seen in tumor cells involves down-regulation of co-stimulatory molecule expression on the cell surface [46]. One reason that the tumor cannot function as an efficient APC may be that it is unable to deliver a co-stimulatory signal (signal 2) that is necessary for productive interactions with T lymphocytes. Down-regulation of

Table 1
Immunosuppressive factors produced by human tumors^a

1. The TNF family ligands: induce leukocyte apoptosis via the TNF family receptors (reviewed in [76])	
FasL	Fas
TRAIL	TRAIL-R
TNF	TNFR1
2. Small molecules	
Prostaglandin E ₂ (PGE ₂)	Inhibits leukocyte functions through increased cAMP [112]
Histamine	Inhibits leukocyte functions through increased cAMP [112]
Epinephrine	Inhibits leukocyte functions through increased cAMP [112]
INOS	Promotes or inhibits Fas-mediated apoptosis by regulation of NO levels [112]
H ₂ O ₂	Has pro-oxidant activity, increases cAMP levels, causes apoptosis in NK cells, inhibits tumor-specific CTL [112]
3. Enzymes	
Indoleamine 2,3-dioxygenase (IDO)	Suppresses T-cell responses [113]
Arginase I	Impairs T-cell functions, decreases ζ chain expression [114]
4. Cytokines	
TGF- β	Inhibits perforin and granzyme mRNA expression; inhibits lymphocyte proliferation [115]
IL-10	Inhibits production of IL-1 β , IFN- γ , IL-12 and TNF α [116,117]
GM-CSF	Promotes expansion of immunosuppressive tumor-associated macrophages [118]
5. Tumor-associated gangliosides	
	Inhibit IL-2 dependent lymphocyte proliferation or induce apoptotic signals [119]

^a A partial list of immunosuppressive factors selected to demonstrate their diversity and a wide spectrum of effects on immune cells.

co-stimulatory molecules, including members of the B7 family [46], on tumor cells leads to unresponsiveness based on MHC-1-restricted antigen presentation without transmission of the critical co-stimulatory signal to leukocytes.

Tumors are also known to directly interfere with the host immune system. They either produce and release factors that modulate functions of immune cells or induce apoptosis of these cells. Table 1 is a partial list of various tumor-derived immunosuppressive factors. As can be seen, even this partial list is long and includes a broad range of biologic effector molecules: several distinct receptor–ligand systems, small molecular species, cellular enzymes, soluble cell components and cytokines/chemokines. This diversity indicates that tumors are incredibly adept in their ability to debilitate host immune responses. Whether all of these factors are selectively employed *in vivo* at various stages of tumor progression or are produced by some tumors but not others has been a subject of intense and continuing debate. For example, it could be readily concluded that more aggressive tumors elaborate several different inhibitory molecules or secrete them at higher levels than less aggressive tumors. However, a formal demonstration of this hypothesis is lacking.

While it is likely that early stages of oncogenesis are accompanied by a combination of tumor-trophic and anti-tumor effects in a large part mediated by infiltrating immune cells, the balance of interactions between the host immune cells and tumor probably changes with time. Once the tumor is established, it begins to orchestrate its escape from the

host immune cells. As indicated above, tumors can evade the host’s interference by being poor stimulators of immune cells as well as by being poor targets, which are not recognized by immune cells. In addition, tumors actively interfere with functions and even survival of immune cells by employing a variety of mechanisms.

5. Mechanisms of tumor evasion

Mechanisms responsible for immune cell dysfunction in patients with cancer are numerous and varied, as illustrated in Fig. 1. In addition to a wide variety of soluble immunosuppressive factors (TGFβ, IL-10, ROS, enzymes, inhibitory ligands such as FasL or TRAIL, as listed in Table 1) that are released by tumor cells or other cells in the tumor microenvironment, suppressor cell populations, *i.e.*, regulatory T cells (CD4+CD25) or myeloid-derived suppressor cells have been shown to play a key role in down-regulation of anti-tumor host immunity [47,48].

Generally, immunosuppressive effects of tumors are best seen locally, at the tumor site. Functional aberrations of TIL freshly isolated from human tumors are well documented in the literature [26]. Most TIL are activated T cells containing variable proportions of CD8+ and CD4+ T cell subsets, which are almost exclusively CD45RO+ memory T cells [26,49]. In comparison to autologous PBL or those isolated from tissues distant from the tumor, TIL have been consistently found

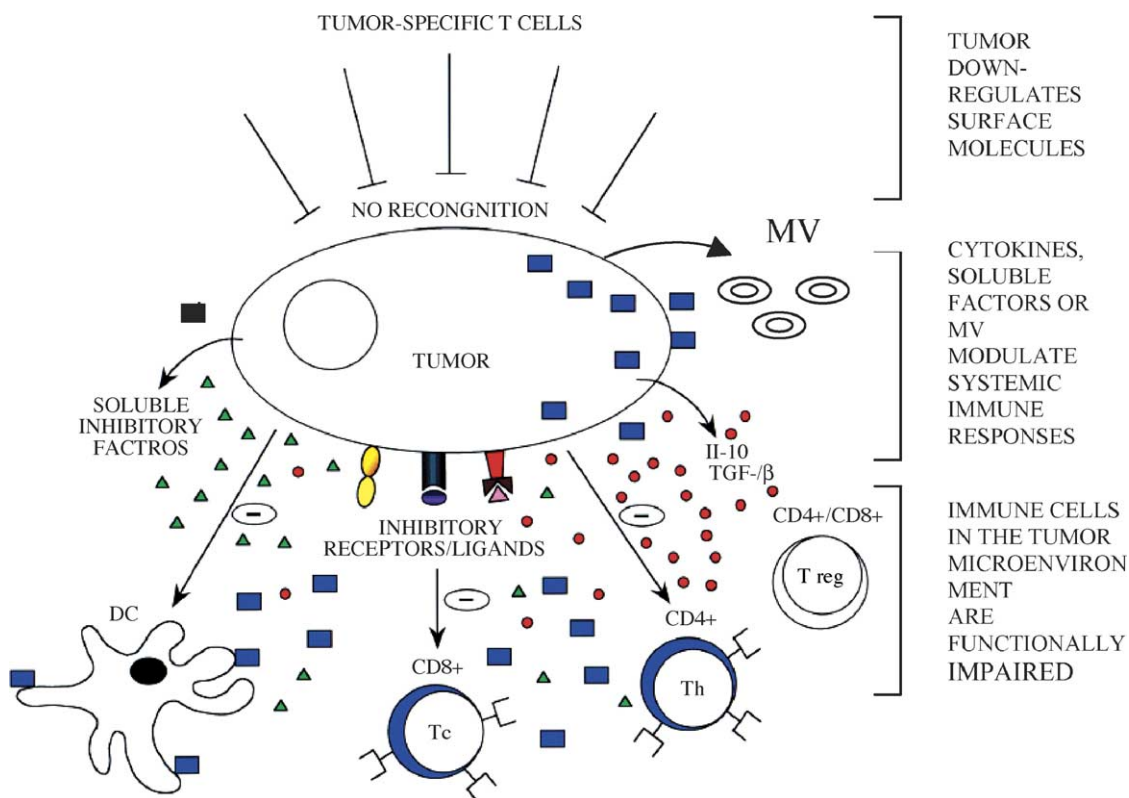


Fig. 1. A schematic representation of various mechanisms responsible for tumor escape. Reproduced with additional details from reference [39].

to be poorly responsive or unresponsive to traditional T-cell activating stimuli [49–51]. While unresponsive to mitogens or antigens, TIL are able to secrete cytokines. However, the profile of cytokines TIL produce may not be equivalent to that in normal T cells. TIL in situ do not produce IL-2 or express IL-2R [52,53], and translation of IL-2 mRNA was found to be defective in TIL isolated from breast carcinoma [52]. It has been suggested that the paucity of Th1 cytokines (i.e., IL-2, IFN- γ and IL-12) at the tumor site or tumor-draining lymph node as well as the prevalence of Treg cytokines (i.e., IL-10 or TGF- β), appear to condition evolving TA-specific T cells toward the less efficacious Th2 or Treg functional phenotypes. In fact, in patients with malignant disease, TIL have been shown to display a predominant Type-2 or T-reg functional phenotype associated with the local production of IL-4 or IL-10, respectively, rather than the mixed Type-1/Type-2 responsiveness observed in normal donors [54]. Therefore, a cytokine imbalance is one of the mechanisms responsible for immune deviation seen at the tumor site.

Alterations in systemic TAA-specific T cell immunity also occur in patients with malignant disease. In the early 1990's Mizoguchi et al. [55], studying dysfunctional T cells from long-term tumor bearing mice, demonstrated a marked decrease in the expression of CD3 ζ chain, and of p56^{lck} as well as p59^{fyn} tyrosine kinases, all of which play a critical role in the signal transduction events that lead to T cell activation [56]. These changes were accompanied by a decreased tyrosine kinase phosphorylation and diminished calcium influx. These findings provided for the first time a molecular basis for T cell dysfunction in cancer patients. More recent studies in patients with malignant disease confirmed these initial observations in murine models. In this regard, T cells and NK cells from approximately half of the patients with carcinoma of the head and neck [56–58], breast [59], colon [60], kidney [61], ovary [62] and prostate [63], non-Hodgkins lymphoma [64], Hodgkins lymphoma [65], cervix [66,67] and melanoma [68,69] demonstrate a decreased CD3 ζ chain expression and a decreased in vitro response to antigens or mitogens. We have also demonstrated that circulating T cells are biased in their cytokine profile or otherwise functionally compromised in patients with malignant disease [70,71]. Importantly, alterations in circulating T cell function, as determined by CD3 ζ chain expression, proliferative index or NF κ B activity, are associated with the extent of alterations in TIL function and with tumor stage [71–73]. These observations suggest that CD3 ζ chain expression may be a marker of immune competence in patients with malignant disease, and that individuals who have normal CD3 ζ chain expression are most likely to respond favorably to biotherapy [56]. It is noteworthy that changes in signal transduction molecules are not limited to CD3 ζ chain. Kolenko et al. demonstrated that JAK-3, a tyrosine kinase associated with the γ chain, a common element to IL2, IL4, IL7 and IL15 cytokine receptors, was also decreased in T cells from RCC patients [74]. Moreover, T cells from RCC patients also had a diminished ability to translocate NF κ Bp65 [70,75].

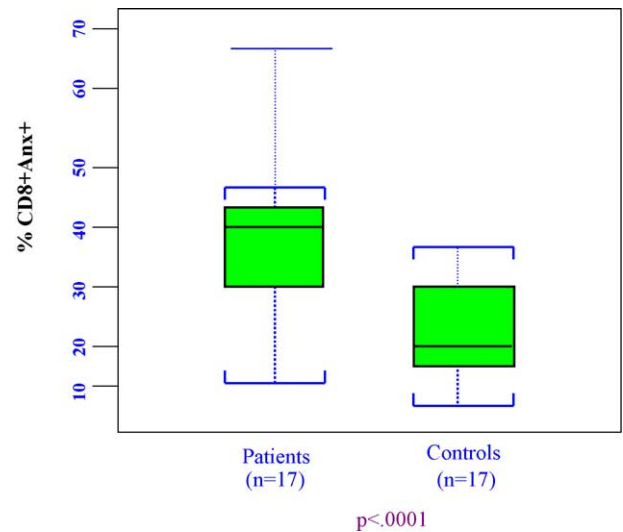


Fig. 2. Circulating CD8+ T cells in patients with cancer bind Annexin V. The data were obtained with freshly-drawn peripheral blood of patients with HNC and age-matched NC by multicolor flow cytometry. A significantly higher proportion of CD3+CD8+ T cells bind Annexin V in the patients than in NC.

Among less known but clearly important immunosuppressive effects tumors mediate is the ability to induce T-cell apoptosis [76]. Studies involving TUNEL staining of TIL and Annexin V binding to circulating T cells suggest that CD8+ rather than CD4+ T cells selectively undergo apoptosis at the tumor site and in the peripheral circulation of patients with cancer [77]. The proportion of CD8+Fas+ T cells that bind Annexin V is significantly increased in the circulation of patients with cancer relative to age-matched normal controls (Fig. 2). Thus, the fate of CD8+ and CD4+ T-cell subsets may differ due to their divergent sensitivity to apoptosis. Also, the effector subpopulations of CD8+ T cells (e.g., CD8+CD45RO+CD27– and CD8+CD28–) appear to be preferentially targeted for apoptosis in patients with cancer [78]. Absolute numbers of circulating T cell subsets are low in patients with cancer [79]. Taken together, these findings suggest that a loss of effector T cell function through targeted apoptosis might severely compromise anti-tumor functions of the host immune system and contribute to tumor progression [77]. It also results in the aberrant lymphocyte homeostasis characterized by a rapid turnover of T cells, especially CD8+ T cells [80].

Different mechanisms may account for the high frequency of T-cell apoptosis observed in patients with cancer. Binding of Fas ligand (FasL) to the Fas receptor has been known for some time to induce apoptosis of T cells responding to autologous antigens and maintain tolerance to normal tissue antigens. Furthermore, chronically stimulated T cells are likely to undergo activation-induced cell death (AICD) mediated by the Fas/FasL pathway, or they may die because appropriate cytokines are not secreted [81]. AICD is induced by repeated or chronic antigenic stimulation, and neither co-stimulatory molecules nor Bcl-2 family members can rescue

T cells from AICD. In this regard, tumor cells produce and secrete a variety of TAA, and TIL, LNL or peripheral T cells in patients with cancer are subject to chronic or repeated antigenic stimulation, and the majority express CD95 on the cell surface [57,82]. Such chronic or acute systemic dissemination of TAA may result in an excess of Ag and “high dose” tolerance of specific T and B cells, making them particularly susceptible to AICD.

A somewhat different but related mechanism may be envisioned, in which the tumor not only induces lymphocyte dysfunction, including the reduced ability to produce IL-2 and IFN- γ [53,83], but also capitalizes on the expression of TNF family receptors on its own surface. A variety of freshly harvested or cultured human tumor cells have been found to express mRNA for FasL as well as surface and/or cytosolic FasL protein (reviewed in [81]). In addition, microvesicles (MV), which are presumably derived from tumor cells and contain biologically active membrane form (42 kDa) of FasL are present in sera of patients with cancer, including those with HNC, ovarian carcinoma and melanoma [84–86]. These structures can mediate apoptosis of Fas+ lymphocytes at sites distant from tumor lesions. Therefore, tumors that express Fas-ligand or shed Fas-ligand-containing MV into serum could induce apoptosis in T cells infiltrating the tumor site as well as circulating T cells, and thus effectively escape from the effector arm of the immune response [76]. On the other hand, Fas-expressing malignant cells are themselves resistant to apoptosis. Some of the mechanisms identified include the over-expression of key anti-apoptotic proteins such as two members of the inhibitors of apoptosis (IAP) family (survivin and ML-IAP), FLICE inhibitory proteins (FLIP), Bcl-2 and the ability to produce inducible nitric oxide synthetases (iNOS) which may play a role in inhibiting apoptosis.

The Fas/FasL pathway inducing receptor- and/or mitochondria-mediated apoptosis of activated T cells [82] is only one example of the receptor–ligand interactions contributing to tumor escape. Inhibitory receptor–ligand pairs known to be involved include members of the B7: CD28 superfamily such as CD28: CTLA-4, ICOS: ICOSL (inducible costimulator) or PD: PD-L1/PD-L2 (program death-1). Receptor–ligand pairs of this superfamily play a key role in regulating T-cell activation and tolerance [87]. Expression of the ligand on tumor cells allows for interactions with its receptor on immune effector cells, inducing functional paralysis or death [87]. Antibodies specific for the ligand on tumor cells have been shown to protect T cells and enhance CTL-mediated tumor death in animal models [87].

An additional mechanism that may also explain the dysfunction, and ultimately, death of T cells in situ might result from the functional impairment in alternate effector cells that accumulate within tumor sites, tumor-associated dendritic cells, and TADC [88,89]. TADC not only process and present TA, but are important sources of IL-1, IL-12, IFN- α , IL-15, IL-18, IL-23 and IL-27, among other cytokines. They are also rich in co-stimulatory molecules (CD80, CD86, OX40, 4-1BBL) necessary as second signals or in growth factors

for T-cell differentiation, proliferation and memory development [89]. Therefore, if TADC are not able to perform normally, as suggested by data in the literature [89], or if they also undergo apoptosis in situ, then TADC–TIL interactions are not likely to be optimal for generating productive TA-specific immunity. The mechanisms responsible for induction of apoptosis and protection of different DC subpopulations and DC precursors from death signals are poorly understood. The molecular pathways that may be involved include: (i) down-regulation of the anti-apoptotic Bcl-2 family proteins in DC [89,90]; (ii) accumulation of ceramides which may interfere with PI3K-mediated survival signals [91], or (iii) production of nitric oxide (NO) species by tumor cells which suppresses expression of cellular inhibitors of apoptosis proteins (cIAPs) [92] or cFLIP. Analysis of gene and protein expression in DC and DC precursors in the tumor microenvironment has demonstrated that expression of several intracellular signaling molecules is reproducibly altered in DC co-incubated with tumor cells, including IRF2, IL-2R γ , Mcl-1, and small Rho GTPases among others [93]. It appears that both intrinsic and extrinsic apoptotic pathways are involved in tumor-induced apoptosis of DC, as determined by an increased resistance to apoptosis of DC genetically-modified to over-express XIAP, Caspase 8, Bcl-xL or FLIP. In addition, recent reports confirm that TADC are functionally defective, especially in their antigen-presenting capacity [94]. This is likely due to defective maturation of DC in the tumor microenvironment, possibly mediated via tumor-derived vascular endothelial growth factor (VEGF) [95]. Alternatively, in vitro demonstration that tumor-derived gangliosides interfere with expression of inducible proteasomal APM components in DC explains yet another mechanism tumors have adapted to facilitate their escape. Moreover, tumor-associated macrophages (TAM) also exhibit functional defects relative to their counterparts in tumor-uninvolved inflammatory lesions in the same patients [96].

Finally, the presence of regulatory immune cells, which are known to accumulate at the tumor site [47] but are also detectable in the peripheral circulation of patients with cancer, has been emphasized as another factor contributing to tumor escape. Lymphoid (CD4+CD25+) and/or myeloid (CD34+ immature, antigen-presenting) suppressor cells down-regulate functions of immune effector cells, presumably via cytokine (IL-10, TGF- β) secretion [97], as part of a normal process of controlling autoimmunity. Because tumors express self-antigens, suppressor cells may be recruited to tumor sites to dampen immune responses to self. In murine models, these cells have been shown to prevent autoimmune disease [98] and to inhibit generation of tumor-specific T-cell responses [97]. Depletion of CD4+CD25+ T cells has been shown to promote tumor rejection in mice [99]. Regulatory cells are best defined by their suppressive function and, thus, in the absence of reliable phenotypic markers are difficult to study in humans. Defined as Foxp3+, CTLA-4+, GITR+ T-cell subsets, they appear to be enriched among TIL in human tumors and are more frequent in the periph-

eral circulation of patients with cancer than of normal donors [100]. Studies are currently in progress to achieve a better understanding of the role and mechanisms of regulatory cell effects on tumor escape from the host immune system.

The mechanisms of escape evolved by human tumors are varied and ingenious. They appear to target components of the innate as well as adaptive immune system; they operate at the local as well systemic levels, and interfere with molecular pathways responsible for the key cellular functions of immune cells. Furthermore, progressing tumors co-opt tissue cells to participate in creating a microenvironment especially unfavorable for immune interventions in situ. As a result of these mechanisms, tumors have become adept at avoiding immune surveillance, and it might be predicted that their escape from the host's immune system is likely to be difficult to overcome by immune therapies.

6. Reversal of immune dysfunction as a goal of cancer immunotherapy

The question of how to best augment and sustain anti-tumor responses during cancer progression has been a focus of biotherapeutic approaches for a long time. Traditionally, cell-mediated biotherapies used in treating cancer patients have been aimed at increasing these responses via activation, amplification of proliferation or re-population of the host with ex vivo activated anti-tumor effector cells. These strategies referred to as active or passive cellular immunotherapy, respectively, have undergone considerable refinement over the years.

Active as well as passive immunotherapy of cancer have the best opportunity to succeed in the setting of minimal residual disease, with elimination of Treg prior to therapy, attenuation of apoptosis of activated tumor-specific T cells and establishment of long-lived anti-tumor memory responses. Also, restoration of normal lymphocyte homeostasis is probably an important component of such immunotherapy. Rosenberg et al. recently treated cancer patients with adoptively transferred autologous ex vivo cultured tumor-specific T cells following lympho-depletion with cyclophosphamide (30–60 mg/kg \times 2 days) plus fludarabine (25 mg/m² \times 5 days) [101,102]. Remarkably, this intentional lympho-depletion of patients before T-cell transfer promoted extensive proliferation of infused T cells, creating an in vivo T-cell repertoire capable of exercising powerful anti-tumor effects and of mediating clinically meaningful tumor regression [101]. In a very recent study, this same strategy was reported to induce clinical responses in 50% of treated patients and documented the persistent presence of tumor-specific T cells in the periphery [102]. These early results indicate that the enhancement of the activity and survival of transferred T cells by previous lympho-depletion is therapeutically effective and mediates durable cancer regression [101,102]. It is possible that this approach takes advantage of endogenous homeostatic mechanisms that restore lym-

phocyte numbers after an episode of lymphopenia. Alternatively, it is possible that elimination of Treg and reversal of their inhibitory effects on the immune system by low-dose cyclophosphamide/fludarabine treatment facilitates survival of transferred T cells. The precise mechanism of these effects is not yet understood but it appears that protection of immune cells and their survival are necessary for achieving therapeutic efficacy. Data are also available in support of cytokine administration, e.g., IL-2, after T-cell transfers or following anti-tumor vaccines in patients with cancer [101,102].

Cytokines play a key role in regulation of lymphocyte survival [103]. The largest amount of experience is with IL-2, which not only prolongs survival of transferred CD8+ T cells but also enhances their anti-tumor activity [104]. In particular, low nontoxic doses of IL-2 administered with transferred antigen-specific T cell lines have been shown to promote in vivo persistence and activity of viral-specific as well as tumor antigen-specific CD8+ T cells [104]. In addition, IL-7, IL-15, IL-12 and IL-21 are currently gaining attention as promising additions to future immunotherapy protocols.

In our hands, cytokines such as IL-2, IL-7, IL-12 and IL-15 are able to rescue activated T cells from tumor-induced killing in vitro. As shown in Fig. 3, these cytokines partially blocked DNA fragmentation in Jurkat cells co-incubated with tumor cells (PCI-13). These cytokines also significantly inhibited Annexin binding to T cells exposed to tumor cell supernatants or to CH-11 agonistic antibody (Fig. 3). The mechanisms of such cytokine-mediated protection of immune cells from tumor-induced effects are not known. We recently observed that expression of CCR7 on CD8+ T cells might be important in protection of these cells from apoptosis [105]. CCR7+CD8+ T cells bind significantly less Annexin V than their CCR7- counterparts and express higher levels of anti-apoptotic Bcl-2 ($p < 0.0001$) but lower levels of pro-apoptotic Bax ($p < 0.01$). Interestingly, patients with HNC whose CD8+ T cells show high spontaneous apoptosis have greatly expanded population of circulating CCR7-CD8- T cells but relatively few CCR7+CD8+ T cells. As the engagement of CCR7 is necessary for protection from spontaneous apoptosis via the PI3K/Akt pathway [105], it is not surprising that CCR7-CD8+ T cells are highly susceptible to apoptosis in these patients. Because CCR7 is also a differentiation marker for CD8+ T cells, its absence on a great majority (e.g., over 80%) of CD8+ T cells in patients with HNC indicates that these cells are in the process of terminal differentiation which is normally followed by apoptosis [106].

A common feature of T cells in the circulation of patients with cancer is low or absent expression of the TCR-associated ζ chain [56]. We have shown previously that down-regulation of ζ chain in T cells may be one manifestation of apoptosis induced by interaction of TIL with FasL+ tumor or by interactions of circulating CD8+ T cells with FasL+ MV [58,107]. Having established a quantitative assay to measure ζ chain expression by flow cytometry, we measured it in patients with cancer before and after immune therapies. As indicated in Fig. 4, expression of ζ chain was significantly

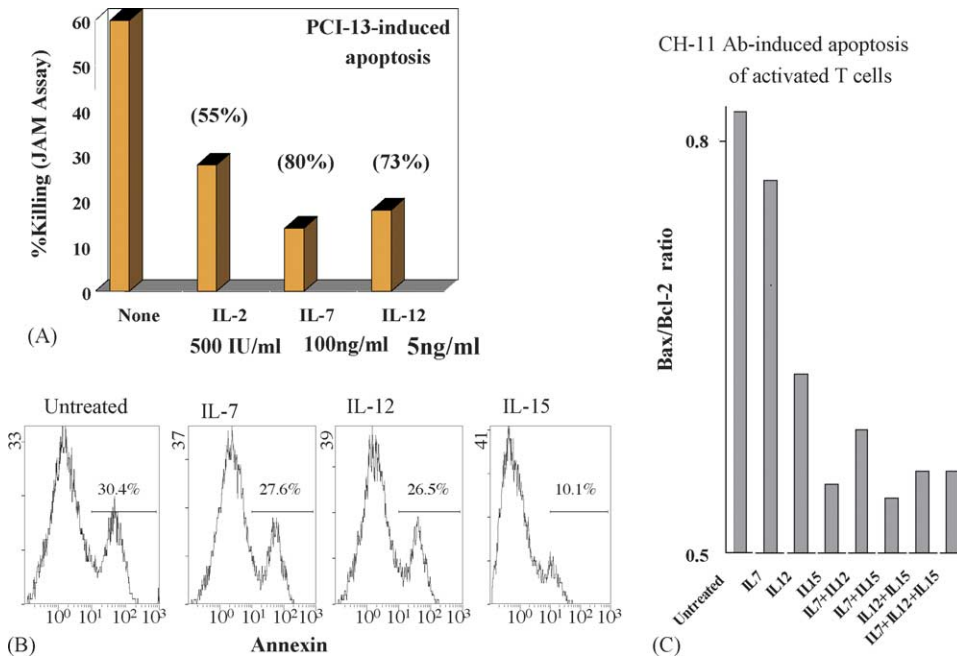


Fig. 3. Protective effects of cytokines on activated T cells. (A) Pre-incubation of activated T cells with cytokines protects them from tumor-induced apoptosis as measured in JAM assays. PCI-13 is a tumor cell line, which induces apoptosis in over 60% of T cells in the absence of cytokines. (B) Annexin V binding to T cells is decreased following their incubation with cytokines prior to exposure to PCI-13 supernatants. (C) A decreased Bax/Bcl-2 ratio is observed by flow cytometry in T cells incubated with cytokines prior to exposure to CH-11 antibody, which induces receptor-mediated apoptosis in activated T cells.

up-regulated in CD8+ T cells of patients with metastatic melanoma treated with IL-2 and histamine dihydrochloride [108]. These results suggest that cytokine therapy tends to restore normal signaling in CD8+ T cells and that this restora-

tion might translate into improved anti-tumor functions and perhaps clinical benefits in these patients. Very preliminary findings in our laboratory also suggest that incubation of freshly-harvested PBMC of cancer patients in the presence of survival cytokines (i.e., IL-2, IL-7, IL-15 or IL-21) tends to diminish their sensitivity to apoptosis, as indicated by lower Annexin binding and a lower Bax/Bcl-2 expression ratio in cytokine-treated vs. untreated CD8+ T cells. Further experiments are clearly needed to establish validity and mechanisms of cytokine-induced protection of activated CD8+ T cells. It is possible that cytokines mediate their cytoprotective effects through receptor-mediated anti-apoptotic signals or through quite distinct effects on differentiation of effector and memory T cells [109]. Evidence has accumulated indicating that common cytokine receptor ζ -chain family $\gamma\text{-}\zeta$ (CD132) cytokines in particular IL-7 and IL-15 act at various stages of the immune response to promote proliferation and survival of T cells [103]. These cytokines appear to be especially important in maintenance of memory CD8+ T cells [110]. Therefore, future vaccination or adoptive T-cell transfers are likely to favor concomitant cytokine therapy with the goals of protecting effector CD8+ T cells from tumor-mediated dysfunction or death and of restoration of normal lymphocyte homeostasis.

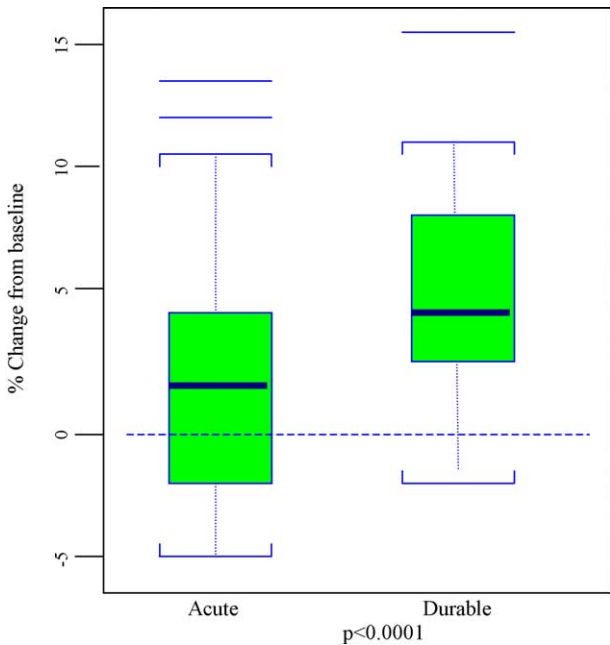


Fig. 4. Expression of ζ in circulating CD8+ T cells after therapy with histamine dihydrochloride and IL-2 in patients with metastatic melanoma (n = 50). Acute and durable effects of therapy on ζ expression relative to baseline expression (stippled line) are illustrated by the boxplots, with medians indicated by solid lines.

7. Conclusions and future prospects

Cancer immunotherapy has now been in the clinic for many years. One reason for its modest therapeutic record to

date is clearly identifiable: tumor-induced deleterious effects on the host immune system have been neglected. These can range from alterations in lymphocyte homeostasis to functional disability or even elimination of effector cell subsets. Tumor-induced effects are persistent and long-lasting. While cancer patients with active and advanced disease usually have the most pronounced immune defects, disease free patients treated with curative therapies may not readily recover immunologic functions. Cancer patients are not immunodeficient, in that their antiviral or antibacterial responses remain undisturbed, however, they are unable to control tumor progression or recurrence. The degree to which the immune system is compromised by the tumor presence is variable, and the most aggressive tumors appear to have evolved multiple mechanisms of escape from the host immune system. Many of these mechanisms target anti-tumor effector cells and interfere with anti-tumor immunity. It is possible that such interference represents tolerance against self-antigens over-expressed by the tumor. It could also represent a failure of lymphocytes to normally differentiate or to survive in the environment re-shaped by the presence of tumor-derived factors and depleted of necessary growth factors, as discussed in the text above.

Over the years, multiple attempts have been made to activate anti-tumor immune responses in patients with cancer. Whether through the delivery of biologic response modifiers, genetic modifications of immune cells or their adoptive transfers, these immunotherapies were all aimed at providing the host with missing activation signals or activated cells capable of exercising anti-tumor activity. One after another, these therapeutic strategies proved to be ineffective. The results of recent anti-tumor vaccination trials, as summarized by Rosenberg et al. [111] make it very clear that active immunotherapy alone is not sufficient to overcome tumor escape. The future immunotherapy of cancer has to be directed not only at stimulation of anti-tumor immune cells but at their protection to assure immune cell survival and maintenance of long-lasting memory. The challenge will be to combine the available therapies, including cancer vaccines, in a way that can best take advantage of insights provided by modern immunology. The various options and strategies open to future immunotherapy of cancer have been eloquently discussed [111]. It is hoped that armed with the knowledge of mechanisms used by the tumor to evade the host immune system, these future therapeutic trials will convincingly demonstrate that immunotherapy can mediate durable cancer regression.

References

- [1] Burnet F. Cancer—a biologic approach. *Br Med J* 1957;1:841–7.
- [2] Thomas L. In: Lawrence HS, editor. Cellular and humoral aspects of the hypersensitive status. New York: Hoeber-Harper; 1959. p. 529–32.
- [3] Penn I. Posttransplant malignancies. *Transplant Proc* 1999;31:1260–2.
- [4] Sheil AG, Disney AP, Mathew TH, Livingston BE, Keogh AM. Lymphoma incidence, cyclosporine, and the evolution and major impact of malignancy following organ transplantation. *Transplant Proc* 1997;29:825–7.
- [5] Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. *Science* 1997;278:1043–50.
- [6] Perera FP. Environment and cancer: who are susceptible? *Science* 1997;278:1068–73.
- [7] Pawelec G, Quayle Q, Colonna-Romano G, et al. Is human immunosenescence clinically relevant? Looking for immunological risk phenotypes. *Trends Immunol* 2002;23:330–2.
- [8] Reiche EMV, Nunes SOV, Morimoto HK. Stress, depression, the immune system and cancer. *Lancet Oncol* 2004;5:617–25.
- [9] Strayer DR, Carter WA, Brodsky I. Familial occurrence of breast cancer is associated with reduced natural killer cytotoxicity. *Breast Cancer Res Treat* 1986;7:187–92.
- [10] Bovbjerg DH, Valdimarsdottir H. Familial cancer, emotional distress, and low natural killer cytotoxic activity in healthy women. *Ann Oncol* 1993;4:745–52.
- [11] Shevde LA, Joshi NN, Shinde SR, Nadkarni JJ. Studies on functional status of circulating lymphocytes in unaffected members from cancer families. *Human Immunol* 1998;59:373–81.
- [12] Reuben JM, Hersh EM. Delayed hypersensitivity responses of cancer patients to recall antigens using a new “multitest” applicator. *Ann Allergy* 1984;53:390–4.
- [13] Ostrand-Rosenberg S. Animal models of tumor immunity immunotherapy and cancer vaccines. *Curr Opin Immunol* 2004;16:1430–50.
- [14] Smyth MJ, Trapani JA. Lymphocyte-mediated immunosurveillance of epithelial cancers? *Trends Immunol* 2001;22:409–11.
- [15] Altman JD, Moss PAH, Boulder PJR, et al. Phenotypic analysis of antigen-specific T lymphocytes. *Science* 1996;274:94–6.
- [16] Meidenbauer N, Hoffmann TK, Donnenberg AD. Direct visualization of antigen-specific T cells using peptide-MHC-class I tetrameric complexes. *Methods* 2003;31:160–71.
- [17] Hoffmann TK, Donnenberg AD, Finkelstein SD, Donnenberg VS, Friebe-Hoffmann F, Myers EN, et al. Frequencies of tetramer+ T cells specific for the wild-type sequence p53_{264–272} peptide in the circulations of patients with head and neck cancer. *Cancer Res* 2002;62:3521–9.
- [18] Palmowski M, Salio M, Dunbar RP, Cerundolo V. The use of HLA class I tetramers to design a vaccination strategy for melanoma patients. *Immunol Rev* 2002;188:155–63.
- [19] Nagorsen D, Scheibenbogen C, Thiel E, Keilholz U. Immunological monitoring of cancer vaccine therapy. *Expert Opin Biol Ther* 2004;4:1677–84.
- [20] Albers AE, Kim GG, Ferris RL, DeLeo AB, Whiteside TL. Immune responses to p53 in patients with cancer enrichment in tetramer+ p53 peptide-specific T cells and regulatory CD4+CD25+ cells at tumor sites. *Cancer Immunol Immunother* 2005;62:670–9.
- [21] Sabin U, Tureci O, Pfreundschuh M. Serologic identification of human tumor antigens. *Curr Opin Immunol* 1997;9:709–16.
- [22] Hoffmann TK, Donnenberg AD, Finkelstein SD, Donnenberg VS, Friebe-Hoffmann U, Myers EN. Frequencies of tetramer+ T cells specific for the wild-type sequence p53_{264–272} peptide in the circulation of patients with head and neck cancer. *Cancer Res* 2002;62:3521–9.
- [23] Matzinger P. An innate sense of danger. *Sem Immunol* 1998;10:399–415.
- [24] Von Kleist S, Berling J, Boxhle W, Wittekind C. Immunohistochemical analysis of lymphocyte subpopulations infiltrating breast carcinomas and benign lesions. *Int J Cancer* 1987;40:18–23.
- [25] Coronella J, Telleman P, Kingsbury, et al. Evidence for an antigen-driven humoral immune response in medullary ductal breast carcinoma. *Cancer Res* 2001;61:7889–99.
- [26] Whiteside TL. Tumor infiltrating lymphocytes in human malignancies. Austin, TX: RG Landes Co.; 1993.

- [27] Whiteside TL. The role of immune cells in the tumor microenvironment. In: Haefner B, editor. *The link between inflammation and cancer*, Kluwer Press, in press.
- [28] Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004;4:540–50.
- [29] Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, et al. Gene expression profiles in normal and cancer cells. *Science* 1997;276:1268–72.
- [30] Bogenrieder T, Herlyn M. Axis of evil: molecular mechanisms of cancer metastasis. *Oncogene* 2003;22:6524–36.
- [31] Nathanson SD. Insights into the mechanisms of lymph node metastasis. *Cancer* 2003;98:413–23.
- [32] Whiteside TL, Herberman RB. The role of natural killer cells in immune surveillance of cancer. *Curr Opin Immunol* 1995;7:704–10.
- [33] Mellstedt H. Monoclonal antibodies in human cancer. *Drugs Today* 2003;39(Suppl.):C1–16.
- [34] Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860–7.
- [35] Akira S, Takeda K. Toll-like receptor signaling. *Nat Rev Immunol* 2004;4:499–511.
- [36] Suganuma M, Okabe S, Marino MW, Sakai A, et al. Essential role of tumor necrosis factor alpha (TNF- α) in tumor promotion as revealed by TNF- α -deficient mice. *Cancer Res* 1999;59:4516–8.
- [37] Dworak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986;315:1650–9.
- [38] Lybarger L, Wang X, Harris M, Hansen TH. Viral immune evasion molecules attack the ER peptide-loading complex and exploit ER-associated degradation pathways. *Curr Opin Immunol* 2005;17:71–8.
- [39] Whiteside TL, Campoli M, Ferrone S. Tumor induced immune suppression and immune escape: mechanisms and possible solutions. In: Nargosen D, Marincola F, editors. *Monitoring T cell directed vaccine trials in cancer patients*, in press.
- [40] Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. *Immunol Res*, in press.
- [41] Meissner M, Reichert TE, Kunkel M, Gooding W, Whiteside TL, Ferrone S, et al. Defects in the HLA class I antigen processing machinery in head and neck squamous cell carcinoma: association with clinical outcome. *Clin Cancer Res* 2005;11:2552–60.
- [42] Hoffmann TK, Nakano K, Elder E, Dworacki G, Finkelstein SD, Apella E, et al. Generation of T cells specific for the wild-type sequence p53_{264–272} peptide in cancer patients—implication for immunoselection of epitope-loss variants. *J Immunol* 2000;165:5938–44.
- [43] Seliger B, Ritz U, Abele R, et al. Immune escape in melanoma: first evidence of structural alterations in two components of the MHC class I antigen processing pathway. *Cancer Res* 2001;61:8647–50.
- [44] Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv Immunol* 2000;74:181–273.
- [45] Campoli M, Chang C-C, Ferrone S. HLA class I antigen loss, tumor immune escape and immune selection. *Vaccine* 2002;20:A40–5.
- [46] Wang S, Chen L. Co-signaling molecules of the B7-CD28 family in positive and negative regulation of T lymphocyte responses. *Microbes Infect* 2004;6:759–66.
- [47] Shevach EM. Fatal attraction: tumors become regulatory T cells. *Nature Med* 2004;10:900–1.
- [48] Gabrilovich DI. Mechanisms and functional significance of tumor-induced dendritic cell differentiation in cancer. *Nat Med* 2004;4:941–52.
- [49] Whiteside TL. Immune responses to malignancies. *J Allergy Clin Immunol* 2003;111:S677–86.
- [50] Whiteside TL. Tumor-infiltrating lymphocytes as antitumor effector cells. *Biotherapy* 1992;5:47–61.
- [51] Sogn JA. Tumor immunology: the glass is half full. *Immunity* 1998;9:757–63.
- [52] Lopez CB, Rao TD, Feiner H, Shapiro R, Marks JR, Frey AB. Repression of interleukin-2 mRNA translation in primary human breast carcinoma tumor-infiltrating lymphocytes. *Cell Immunol* 1998;190:141–55.
- [53] Rabinowich H, Suminami Y, Reichert TE, Crowley-Nowich P, Bell M, Whiteside TL. Expression of cytokine genes or proteins and signaling molecules in lymphocytes associated with human ovarian Carcinoma. *Int J Cancer* 1996;68:276–84.
- [54] Ochoa A, editor. *Mechanisms of tumor escape from the immune response*. London and New York: Taylor and Francis; 2003.
- [55] Mizoguchi H, O’Shea JJ, Longo DL, Loeffler CM, McVicar DW, Ochoa A. Alterations in signal transduction molecules in T lymphocytes from tumor bearing mice. *Science* 1992;258:1795–8.
- [56] Whiteside TL. Down-regulation of ζ chain expression in T cells: a biomarker of prognosis in cancer? *Cancer Immunol Immunother* 2004;53:865–76.
- [57] Reichert TE, Rabinowich H, Johnson JT, Whiteside TL. Human immune cells in the tumor microenvironment: mechanisms responsible for signaling and functional defects. *J Immunother* 1998;21:295–306.
- [58] Reichert TE, Day R, Wagner E, Whiteside TL. Absent or low expression of the ζ chain in T cells at the tumor site correlates with poor survival in patients with oral carcinoma. *Cancer Res* 1998;58:5344–7.
- [59] Kurt RA, Urba WJ, Smith JW, Schoof DD. Peripheral T lymphocytes from women with breast cancer exhibit abnormal protein expression of several signaling molecules. *Int J Cancer* 1998;78:16–20.
- [60] Nakagomi H, Petersson M, Magnusson I, Juhlin C, Matsuda M, Mellstedt H, et al. Decreased expression of the signal-transducing ζ chains in tumor-infiltrating T-cell and NK cells of patients with colorectal carcinoma. *Cancer Res* 1993;53:5610–2.
- [61] Finke JH, Zea AH, Stanley J, Longo DL, Mizoguchi H, Tubbs RR, et al. Loss of T-cell receptor ζ chain and p56^{lck} in T-cell infiltrating human renal cell carcinoma. *Cancer Res* 1993;53:5613–6.
- [62] Lai P, Rabinowich H, Crowley-Nowick PA, Bell MC, Mantovani G, Whiteside TL. Alterations in expression and function of signal transduction proteins in tumor associated NK and T lymphocytes from patients with ovarian carcinoma. *Clin Cancer Res* 1996;2:161–73.
- [63] Meidenbauer N, Gooding W, Spitzer L, Whiteside TL. Recovery of ζ chain expression and changes in spontaneous IL-10 production after PSA-based vaccines in patients with prostate cancer. *Br J Cancer* 2002;86:168–78.
- [64] Wang Q, Stanley J, Kudoh S, Myles J, Kolenko V, Yi T, et al. T cells infiltrating non-Hodgkin’s B cell lymphomas show altered tyrosine phosphorylation pattern even though T cell receptor/CD3-associated kinases are present. *J Immunol* 1995;155:1382–92.
- [65] Frydecka I, Kaczmarek P, Bocko D, Kosmaczewska A, Morilla R, Catovsky D. Expression of signal-transducing zeta chain in peripheral blood T cells and natural killer cells in patients with Hodgkin’s disease in different phases of the disease. *Leuk Lymphoma* 1999;35:545–54.
- [66] de Gruijl TD, Bontkes HJ, Peccatori F, Gallee MP, Helmerhorst TJ, Verheijen RH, et al. Expression of CD3-zeta on T-cells in primary cervical carcinoma and in metastasis-positive and -negative pelvic lymph nodes. *Br J Cancer* 1999;79:1127–32.
- [67] Kono K, Rensing ME, Brandt RM, Melief CJ, Potkul RK, Anderson B, et al. Decreased expression of signal-transducing zeta chain in peripheral T cells and natural killer cells in patients with cervical cancer. *Clin Cancer Res* 1996;2:1825–8.
- [68] Zea AH, Cutri BD, Longo DL, Alvord WG, Strobl SL, Mizoguchi H, et al. Alterations in T cell receptor and signal transduction molecules in melanoma patients. *Clin Cancer Res* 1995;1:1327–35.

- [69] Rabinowich H, Banks M, Reichert T, Logan TF, Kirkwood JM, Whiteside TL. Expression and activity of signaling molecules in T lymphocytes obtained from patients with metastatic melanoma before and after IL-2 therapy. *Clin Cancer Res* 1996;2:1263-74.
- [70] Uzzo R, Rayman P, Kolenko V, Clark PE, Cathcart MK, Bloom T, et al. Renal cell carcinoma-derived gangliosides suppress nuclear factor- κ B activation in T cells. *J Clin Invest* 1999;104:769-76.
- [71] Rabinowich H, Suminami Y, Reichert TE, Crowley-Nowich P, Bell M, Whiteside TL. Expression of cytokine genes or proteins and signaling molecules in lymphocytes associated with human ovarian carcinoma. *Int J Cancer* 1996;68:276-84.
- [72] Reichert TE, Strauss L, Wagner EM, Gooding W, Whiteside TL. Signaling abnormalities and reduced proliferation of circulating and tumor-infiltrating lymphocytes in patients with oral carcinoma. *Clin Cancer Res* 2002;8:3137-45.
- [73] Bukowski RM, Rayman P, Uzzo R, Bloom T, Sandstrom K, et al. Signal transduction abnormalities in T lymphocytes from patients with advanced renal cell carcinoma: clinical relevance and effects of cytokine therapy. *Clin Cancer Res* 1998;4:2337-47.
- [74] Kolenko V, Wang Q, Riedy MC, O'Shea J, Ritz J, Cathcart MK, et al. Tumor-induced suppression of T lymphocyte proliferation coincides with inhibition of Jak3 expression and IL-2 receptor signaling: role of soluble products from human renal cell carcinomas. *J Immunol* 1997;159:3057-67.
- [75] Uzzo RG, Clark PE, Rayman P, Bloom T, Rybicki L, Novick A, et al. Evidence that tumor inhibits NF κ B activation in T lymphocytes of patients with renal cell carcinoma. *J Natl Cancer Inst* 1999;91:718-21.
- [76] Whiteside TL. Tumor-induced death of immune cells: its mechanisms and consequences. *Semin Cancer Biol* 2002;12:43-50.
- [77] Hoffmann TK, Dworacki G, Meidenbauer N, Gooding W, Johnson JT, Whiteside TL. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. *Clin Cancer Res* 2002;8:2553-62.
- [78] Whiteside TL. Apoptosis of immune cells in the tumor microenvironment and peripheral circulation of patients with cancer: implications for immunotherapy. *Vaccine* 2002;20:A46-51.
- [79] Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2004;10:3755-62.
- [80] Kuss I, Schaefer C, Godfrey TE, Ferris RL, Harris J, Gooding W, Whiteside TL. Recent thymic emigrants and subsets of naive and memory T cells in the circulation of patients with head and neck cancer. *Clin Immunol* 2005;116:27-36.
- [81] Van Parijs L, Ibrahimov A, Abbas AK. The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* 1996;93:951-5.
- [82] Kim W-J, Tsukishiro T, Johnson JT, Whiteside TL. Expression of pro- and anti-apoptotic proteins in circulating CD8+ T cells of patients with squamous cell carcinoma of the head and neck. *Clin Cancer Res* 2004;10:5101-10.
- [83] Vitolo D, Zerbe T, Kanbour A, Dahl C, Herberman RB, Whiteside TL. Expression of mRNA for cytokines in tumor-infiltrating mononuclear cells in ovarian adenocarcinoma and invasive breast cancer. *Int J Cancer* 1992;51:573-80.
- [84] Andreola G, Rivoltini L, Castelli C, Huber V, Perego P, Deho P, et al. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. *J Exp Med* 2002;195:1303-16.
- [85] Taylor DD, Gercel-Taylor C, Lyons KS, Stanson J, Whiteside TL. T-cell apoptosis and suppression of T-cell receptor/CD3- ζ by Fas Ligand-containing membrane vesicles shed from ovarian tumors. *Clin Cancer Res* 2003;9:5113-9.
- [86] Kim J-W, Wieckowski E, Taylor DD, Reichert TE, Watkins S, Whiteside TL. FasL+ membrane vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. *Clin Cancer Res* 2005;11:1010-20.
- [87] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nature med* 2002;8:793-800.
- [88] Shurin MR, Esche C, Lokshin A, Lotze MT. Tumors induce apoptosis of dendritic cells in vitro. *J Immunother* 1997;20:403.
- [89] Gabilovich DI. Mechanisms and functional significance of tumor-induced dendritic cell differentiation in cancer. *Nat Med* 2004;4:941-52.
- [90] Whiteside TL, Odoux C. Dendritic cell biology and cancer therapy. *Cancer Immunol Immunother* 2004;53:240-8.
- [91] Pirtskhalaishvili G, Shurin GV, Esche C, Salup RR, Lotze MT, Shurin MR. Cytokine-mediated protection of human dendritic cells from prostate cancer-induced apoptosis is regulated by the Bcl-2 family of proteins. *Br J Cancer* 2000;83:506-13.
- [92] Esche C, Shurin GV, Kirkwood JM, Wang GQ, Rabinowich H, Pirtskhalaishvili G, et al. TNF- α -promoted expression of Bcl-2 and inhibition of mitochondrial cytochrome c release mediated resistance of mature dendritic cells to melanoma-induced apoptosis. *Clin Cancer Res* 2001;7:974s-979s.
- [93] Pirtskhalaishvili G, Gambotto A, Esche C, Yurkovetsky ZR, Lotze MT, Shurin MR. IL-12 and Bcl-x1 gene transfection of murine dendritic cells protects them from prostate cancer-induced apoptosis and improves their antitumor activity. *J Urol* 2000;163:105.
- [94] Gabilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP. Decreased antigen presentation by dendritic cells in patients with breast cancer. *Clin Cancer Res* 1997;3:483-90.
- [95] Gabilovich DI, Chen HL, Girgis KR, Cunningham T, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nature Med* 1996;2:1096-103.
- [96] Mantovani A. Tumor-associated macrophages in neoplastic progression: a paradigm for the in vivo function of chemokines. *Lab Invest* 1994;71:5-16.
- [97] Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;10:942-9.
- [98] Shevach EM. Regulatory T cells in autoimmunity. *Annu Rev Immunol* 2000;18:423-49.
- [99] Gallimore A, Sakaguchi S. Regulation of tumour immunity by CD25+ T cells. *Immunology* 2002;107:5-9.
- [100] Schaefer C, Kim GG, Albers A, Hoermann K, Myers EN, Whiteside TL. Characteristics of CD4+CD25+ regulatory T cells in the peripheral circulation of patients with head and neck cancer. *Br J Cancer* 2005;92:913-20.
- [101] Dudley EM, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients following clonal repopulation with antitumor lymphocytes. *Science* 2002;298:850-4.
- [102] Dudley EM, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005;23:2346-57.
- [103] Schluns KS, Lefrancois L. Cytokine control of memory T-cell development and survival. *Nature Rev* 2003;3:269-79.
- [104] Van Parijs L, Refaeli Y, Lord JD, Nelson BH, Abbas AK, Baltimore D. Uncoupling IL-2 signals that regulate T cell proliferation, survival, and Fas-mediated activation-induced cell death. *Immunity* 1999;11:281-8.
- [105] Kim J-W, Ferris RL, Whiteside TL. Chemokine receptor 7 (CCR7) expression and protection of circulating CD8+ T lymphocytes from apoptosis. *Clin Cancer Res*, in press.
- [106] Sallusto F, Lenig D, Foster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potential and effector functions. *Nature* 1999;401:708-12.
- [107] Taylor DD, Bender DP, el-Taylor GEI, Stanson J, Whiteside TL. Modulation of TcR/CD3-zeta chain expression by a circu-

- lating factor derived from ovarian cancer patients. *Br J Cancer* 2001;84:1624–9.
- [108] Whiteside T, Gooding W, Elder E, Stover L, Glaspy J, McMasters K, et al. Immunomodulatory effects of combination therapy with histamine dihydrochloride and interleukin-2 during a phase II study in stage IV melanoma. In: *Proceedings of the 17th Annual Scientific Meeting of the Society for Biological Therapy, La Jolla, CA* [Abstract no. 63]; 2002.
- [109] Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nature Rev* 2002;2:251–62.
- [110] Marrack P, Kappler J. Control of T cell viability. *Annu Rev Immunol* 2004;22:765–87.
- [111] Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. *Nature Med* 2004;10:909–15.
- [112] Uotila P. The role of cyclic AMP and oxygen intermediates in the inhibition of cellular immunity in cancer. *Cancer Immunol Immunother* 1996;43:1–9.
- [113] Grohmann U, Fallerino F, Pucetti P. Tolerance, DC and tryptophan: much ado about IDO. *Trends Immunol* 2003;24:242–8.
- [114] Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* 2004;64:5839–49.
- [115] Akhurst RJ, Derynck R. TGF-beta signaling in cancer: a double-edged sword. *Trends Cell Biol* 2001;11:44–51.
- [116] Mocellin S, Marincola F, Rossi CR, Nitti D, Lise M. The multifaceted relationship between IL-10 and adaptive immunity: putting together the pieces of a puzzle. *Cytokine Growth Factor Rev* 2004;15:61–76.
- [117] Vicari AP, Trinchieri G. Interleukin-10 in viral diseases and cancer: exiting the labyrinth. *Immunol Rev* 2004;202:223–36.
- [118] Young MR. Trials and tribulations of immunotherapy as a treatment option for patients with squamous cell carcinoma of the head and neck. *Cancer Immunol Immunother* 2004;53:375–82.
- [119] McKallip R, Li R, Ladisch S. Tumor gangliosides inhibit the tumor-specific immune response. *J Immunol* 1999;163:3718–26.