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.

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

Contents

1. Host immune competence and cancer ................................................................................................. 0
2. Is tumor progression helped by immune cells? .................................................................................. 0
3. Inflammation and cancer .................................................................................................................... 0
4. How tumors evade the host immune system ..................................................................................... 0
5. Mechanisms of tumor evasion ........................................................................................................... 0
6. Reversal of immune dysfunction as a goal of cancer immunotherapy .............................................. 0
7. Conclusions and future prospects .................................................................................................... 0

References

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...
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,
to the arising or progressing tumor is conflicted by evidence indicating immune cells, to subserve tumor needs. More aggressive tumors might be more successful in subverting the microenvironment, including that delivered by the tumor. More aggressive tumors might be able to mediate antibody-dependent cellular cytotoxicity (ADCC) if 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].

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 that delivered by the tumor. More aggressive tumors might be able to modulate their own microenvironment and recruit tumor-favoring immune cells through 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., 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 asbestos 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 (MØ), and induce massive release of ROS, chemokines, cytokines and enzymes from MØ 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 knockout (KO) mice have shown that pro-inflammatory cytokines, e.g., TNF-α, play a key role in inducing carcinogenesis, probably acting via the NFκB 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 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-β2m-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[36-272], 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

<table>
<thead>
<tr>
<th>Immunosuppressive factors produced by human tumors*</th>
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<tr>
<td><strong>1. The TNF family ligands: induce leukocyte apoptosis via the TNF family receptors (reviewed in [76])</strong></td>
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<td><strong>2. Small molecules</strong></td>
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<td>H2O2</td>
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<td><strong>3. Enzymes</strong></td>
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<td>Arginase I</td>
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<td><strong>4. Cytokines</strong></td>
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<td>GM-CSF</td>
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<td><strong>5. Tumor-associated gangliosides</strong></td>
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* 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](image_url). 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 Treg 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 CD3ζ chain, and of p56lck as well as p59fyn 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 CD3ζ 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 CD3ζ chain expression, proliferative index or NFkβ activity, are associated with the extent of alterations in TIL function and with tumor stage [71–73]. These observations suggest that CD3ζ chain expression may be a marker of immune competence in patients with malignant disease, and that individuals who have normal CD3ζ chain expression are most likely to respond favorably to biotherapy [56]. It is noteworthy that changes in signal transduction molecules are not limited to CD3ζ chain. Kolenko et al. demonstrated that JAK-3, a tyrosine kinase associated with the γ chain, a common element to IL-2, IL-4, IL-7 and IL-15 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 NFκBp65 [70,75].

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+ 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 effectorsubpopulations 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].

Differences in circulating 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 family such as CD28: CTLA-4, ICOS: ICOSL, (inducible co-stimulator) 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 interaction with its receptor on immune effector cells, inducing functional paralysis or death [87]. Antibodies specific for the Fas 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 functions 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 in or 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-2Rγ, 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 proapoptotic 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 (CD14+ 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-
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 therapies 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 × 2 days) plus fludarabine (25 mg/m² × 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 lymphocyte 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 V 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
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 restoration 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−γc (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.

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 homoeostasis 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 undiminished; 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 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 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 responses in patients with cancer.

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