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REVIEW

Rolf Kiessling · Ken Wasserman · Shigetoshi Horiguchi
Koji Kono · Jan Sjöberg · Pavel Pisa · Max Petersson

Tumor-induced immune dysfunction

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Abstract Immune system-based approaches for the treatment of malignant disease over the past decades have often focused on cytolytic effector cells such as cytotoxic T lymphocytes (CTL), and natural killer (NK) cells. It has also been demonstrated that tumor-bearing mice can be cured using a wide variety of approaches, some of which involve cytokine-mediated enhancement of CTL and NK cell activity. However, the apparent success in mice stands in contrast to the current situation in the clinic, wherein only a minority of patients have thus far benefited from CTL- or NK cell-based antitumor approaches. The underlying causes of tumor-associated immune suppression of CTL and NK cell activity are discussed, and features of interest shared with HIV infection, leprosy, and rheumatoid arthritis are also be mentioned. Remarkable and very recent observations have shed more light upon the causes of dysfunctional alterations in CTL and NK cells often associated with these diseases, that in turn have suggested new immunotherapeutic approaches for cancer and infectious disease.

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R. Kiessling (✉) · S. Horiguchi · P. Pisa · M. Petersson
Cancer Center Karolinska, Immune- and Genetherapy Laboratory, KS-ringen R8:01, Karolinska Hospital, S-171 76 Stockholm, Sweden
e-mail: Rolf.Kiessling@mtc.ki.se;
Fax: +46-8-309195

K. Wasserman
LMI, DBS, NCI-Frederick Cancer Research and Development Center, Bldg. 560, Frederick, MD 21702, USA

K. Kono
Yamanashi University Medical School, Department of Surgery, 1110 Shimogato, Tamaho-Cho, Nakagoma-Gun, Yamanashi-Ken, 409-38, Japan

J. Sjöberg
Department of Hematology and Infectious Diseases, Karolinska Hospital, S-171 76 Stockholm, Sweden

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Introduction

It is well established that cancer patients or experimental animals with large tumors may have a defective immune system which is secondary to their disease, and immune dysfunctions associated with the presence of progressively growing tumors might explain the often disappointing results obtained thus far from many ongoing clinical trials based on active immunotherapy. Whereas the early stages of experimental tumor growth appear not to elicit systemic immune deficiency [86] and are sometimes associated with antigen-specific tolerance [77], generalized immunodeficiency can arise during the late stages of tumor development [62]. It is the aim of the present article to stress the differences as well as the possible relatedness between these two types of tumor-induced immune suppression. As the latter type of generalized immune suppression seems to predominate in patients with advanced cancer, a better understanding of its nature and of its underlying mechanisms will be of importance in relation to ongoing efforts to employ immunotherapy for cancer in the clinic.

Tumor antigen-specific tolerance or local nonresponsiveness explains the inability of T cells to respond to tumor antigens during the initial growth of transplantable tumors

Early during the growth of experimental tumors, the host immune system responses to nonspecific antigens such as alloantigens seem to be normal [86], and more subtle alterations appear to explain the apparent enigma of how an antigenic tumor can survive in an animal with an intact immune system. The mechanisms involved in the inability of tumor-specific $CD4^+$ T cells and $CD8^+$

T cells to respond to tumor antigens appear to be different. For $CD4^+$ T cells, an antigen-specific immune dysfunction seems to dominate during the initial stages of tumor growth, as has been shown using a "self" tumor antigen model [75]. Perhaps mechanisms similar to those operating to prevent damage to self antigens expressed by normal tissues induce an antigen-specific tolerance, causing the blocking of an effective immune response to tumor antigens.

After adoptive transfer of T cell receptor (TCR) transgenic T cells specific for a model tumor antigen, progressive induction of antigen-specific anergy among $CD4^+$ T cells following injection of tumor cells expressing the cognate antigen has been observed [77]. Isolated transgenic $CD4^+$ T cells from tumor-bearing hosts do not proliferate or secrete cytokines in response to cognate peptide-pulsed antigen-presenting cells. Since the A20 lymphoma used in this study was positive for the B7 costimulatory molecule, this would argue against the simple interpretation that B7 expression determines whether immunity to tumors will be activated or tolerance induced. Recently, Qin et al. [66] have suggested another mechanism for the induction of tolerance in $CD4^+$ antitumor immunity. They demonstrated improved antitumor immunity in mice lacking B cells, and suggested that presentation of tumor antigens by B cells to $CD4^+$ T cells results in a Th2-type humoral immune response in place of the Th1 response required for efficient tumor rejection. Thus, a lack of antigen-specific $CD4^+$ T cell help due to clonal exhaustion or improper antigen presentation might explain $CD4^+$ T cell nonresponsiveness during the initial phases of tumor growth in experimental models.

While it thus appears that the induction of tumor antigen-specific $CD4^+$ T cell nonresponsiveness may involve exhaustion or anergy or improper antigen presentation, evidence indicates that tolerance induction in $CD8^+$ T cells during tumor progression is more passive. It has recently become possible to answer the important question as to whether the T cells that remain capable of rejecting normal grafts in tumor-bearing animals and the cells that fail to reject tumors carrying the same antigens are members of the same population or of different populations. This was approached by using TCR transgenic mice possessing $CD8^+$ cells specific for a class I antigen [86] or for a class I-restricted epitope of the LCMV virus glycoprotein [34].

In the model of Wick et al. [86], tumors expressing a particular class I antigen are not rejected, whereas normal grafts are. This shows that the same cells that fail to reject the tumor can reject antigenically identical normal tissue, i.e. they have not been deleted, anergized or clonally exhausted. Wick et al. further showed that tumor rejection is seen only if the host animals are presensitized to CD80-transfected parental tumor cells and skin grafts, but that even these strong stimuli do not result in rejection of established tumors [86]. In the TCR transgenic model studied by Hermans et al. [34], the Lewis lung carcinoma transfected with a minigene of an LCMV glycoprotein epitope grows unhindered even if greater than 50% of circulating $CD8^+$ T cells are specific for the tumor. Yet when these mice are immunized with dendritic cells (DC) loaded with the specific LCMV epitope, the TCR transgenic T cells are activated as shown by a switch of TCR transgenic T cells from a $CD44^{low}$ to a $CD44^{high}$ phenotype and an increased capacity to produce IFN-γ in vitro, and as a result an antitumor immune response is initiated.

These models stress that tumor cells can survive in the face of a normal immune response even when the majority of T cells are tumor specific and suggest that the functional T cells specific for antigens expressed by the tumor are nonetheless unable to reject it. This supports the notion that tumors create immunoprivileged sites and are protected from local T cell activation. Multiple mechanisms may explain this local nonresponsiveness of T cells in tumors. The local ignorance and failure to activate antitumor immune responses early during tumor progression may share mechanisms with local ignorance of autoreactive T cells to react with self antigens, as studied, for example, in mice expressing the LCMV glycoprotein in beta islet cells of the pancreas. These mice do not develop autoimmune diabetes unless infected with LCMV [35, 60]. Expression of a self antigen or a tumor antigen on a cell type without strong antigen-presenting capabilities in the tumor or of autoantigens in organs such as the pancreas may thus result in ignorance rather than response or anergy. Alternatively, the T cells are locally eliminated or functionally incapacitated by mechanisms that later during tumor growth may also lead to a systemic and more generalized immune suppression, as will be argued below.

It is notable, however, that all the above cited experimental models are from transplantable mouse tumors which grow relatively rapidly and bear little resemblance to slowly progressing human tumors, which as will be argued below mainly induce a nonspecific and systemic immune dysfunction.

Nonspecific and systemic immune dysfunctions are prevalent in late-stage cancer

In contrast to the antigen-specific immune dysfunction during the early stages of tumor growth, as observed mainly in mouse experimental systems, advanced cancer is often associated with a more global change in the immune system that is not specific for the antigens expressed by the tumor. Although generalized immune dysfunctions are often induced by administration of antineoplastic drugs, radiation therapy, or blood or platelet transfusions or are secondary to nutritional deficiencies or aging [9, 15], a substantial degree of tumor-induced immune suppression can only be accounted for by processes induced by the growth of the malignant tumor itself.

Clearly, the degree of immunosuppression is more pronounced in certain types of tumors than in others, with hematopoietic malignancies and primary brain tu-

mors probably being the most frequently cited examples of a malignant disease inducing immune suppression. Immune suppression in hematological malignancies, such as chronic lymphocytic leukemia (CLL), has been explained mainly in terms of replacement of normal hematopoietic cells in the bone marrow by leukemic cells. It has, however, become increasingly clear that it is not only the numbers but above all the function of the B and T cells that is impaired [6, 47]. Hodgkin's disease (HD) is an example of another hematological malignancy associated with both a local and a general immune suppression [23, 83]. HD is characterized by the presence of only a very small number of the typical Hodgkin and Reed-Sternberg tumor cells. A generalized defect in the expression of the TCR zeta chain has also been observed in the peripheral blood of these patients [70]. The local immunosuppression within the HD tumor may be attributed not only to this "global" reduction of TCR zeta expression, but also probably to other underlying mechanisms (J. Sjöberg, in preparation).

In myeloid malignancies the function of T cells and natural killer (NK) cells has also been shown to deteriorate with transformation from preleukemia to advanced disease, and this has recently been shown to be associated with loss of expression of TCR zeta and associated protein tyrosine kinases [10].

Patients with primary intracranial tumor, even those with relatively small tumor burdens, also show a broad range of immunologic defects with manifestations ranging from poor delayed-type hypersensitivity (DTH), lymphopenia, depressed Ig production and impaired lymphocyte responsiveness [17, 54]. This hyporesponsiveness is confined mainly to the $CD4^+$ T cell subset. The mechanisms may be related to an inability of stimulated T cells to express sufficient levels of the alpha chain of the IL-2R in their plasma membrane and leading to losses in IL-2 responsiveness [17]. Following stimulation of the TCR/CD3 complex on these patients' T cells, defects in early transmembrane signaling are seen, including a marked decrease in the tyrosine phosphorylation of a number of proteins [54].

Although hematological malignancies and brain tumors are commonly recognized as being immunosuppressive, it is becoming increasingly clear that the majority of common solid tumors in their advanced stages also have detrimental effects on the immune system. Both in vivo and in vitro methods have been used to detect immune abnormalities in cancer patients. In vivo, the DTH reaction, which is measured by intradermal skin tests with various recall antigens derived from common microbial agents, is often regarded as a measure of cell-mediated immunity in humans [72]. In patients with several different types of hematological and solid tumors, DTH skin test reactivity has been shown to correlate inversely with stage of the disease [14, 43, 55] and also to provide prognostic information [9, 14, 36].

In vitro, defects in the production of Th1 cytokines and in cytotoxic and proliferative functions of T cells and NK cells from cancer patients have been detected, and these frequently correlate with alterations in signal-transducing molecules. A decline in IFN-γ and IL-2 production following anti-CD3 stimulation of T cells from melanoma patients correlates with decreased TCR zeta levels and an adverse disease prognosis [89]. Impaired production of IFN-γ has been found in whole blood cell cultures from patients with urinary bladder carcinoma, renal cell carcinoma (RCC) and colorectal carcinoma as compared to benign tumor-bearing and normal controls [18–21, 41, 80]. Furthermore, cytotoxic T lymphocyte (CTL) responses to influenza virus are deficient in patients with cervical cancer or melanomas [71, 74], which are also correlated with decreased expression of TCR zeta [41, 58], and mice with primary chemically induced tumors have a severely reduced capacity to generate influenza-specific CTL [38].

More direct evidence for cytokine release abnormalities via changes in signal transduction components has come from studies of RCC and ovarian carcinoma tumor-infiltrating lymphocytes (TIL). Impaired TNFα production, after stimulation of RCC TIL with PHA or anti-CD3, and a positive relationship between TNFα induction and zeta-chain levels have been found [80]. Factors other than decreased TCR zeta expression are also involved, however, since several TILs with normal zeta content do not secrete TNFα after mitogenic stimulation. Interestingly, production of IL-2 and IFN-γ was, in this study, unaltered in TIL versus normal peripheral blood lymphocytes (PBL). Alexander et al. [2] have also found normal IL-2 production in the face of impaired proliferative capacity of RCC TIL. These and the findings of others [84], showing that a TCR/CD3 complex with a transduction-defective zeta dimer is still able to trigger IL-2 release in response to anti-CD3 monoclonal antibody (mAb), indicate that signal transduction can perhaps be mediated via functional units other than TCR zeta.

Other observations also support the interpretation that not all effector functions activated via TCR/CD3 are indiscriminately impaired in T cells from cancer patients. Ovarian carcinoma tumor-associated T cells (TAL-T) retain their ability to produce IL-10 following stimulation with anti-CD3 mAb, whereas IFN-γ release is reduced and alterations in signal-transducing molecules are found [44]. These observations and the abundant expression of IL-10 mRNA in ovarian carcinoma [64] also indicate that the presence of this Th2 cytokine, in the absence of co-stimulation, might foster tumor escape. In addition, IL-10 secretion levels from the PBL of patients with cervical intraepithelial neoplasia (CIN) and cervical carcinoma are normal despite reduced TCR zeta and CD16 zeta levels, IFN-γ and TNF production after anti-CD3 stimulation of these PBL is also significantly impaired [41, 58], thus arguing for a selective Th1 defect. Functional impairment of T cells which do not show any decrease in TCR-zeta have been found in patients with early breast carcinoma [58], indicating that defects other than low TCR-zeta expression can give rise to poorly functioning T cells in cancer patients.

Along these lines it is noteworthy that there has been an explosion of knowledge regarding signal transduction mechanisms that transduce cytokine signals dependent upon Janus family tyrosine kinases (JAKs) and cytosolic latent transcription factors known as signal transducers and activators of transcription (STATs) [45]. These signal transduction elements act downstream from cell surface receptors critical for normal T and NK cell functions. T and B cells from mice bearing subcutaneous mammary adenocarcinoma tumors have been found to be defective as a consequence of a marked decrease in STAT-5a content, whereas levels of the TCR and STAT-1, -3, and -6 are normal [63]. Defects in cytotoxic functions are not confined to the adaptive immune system, since NK cells from patients with advanced tumors also function poorly [39]. This loss is also correlated with reduced expression of signal-transducing molecules in patients with colorectal or ovarian carcinomas [44, 48]. Although antitumor NK cytotoxicity is not strictly dependent on CD16 [3], recent reports suggest that cytolytic triggering receptors on NK cells, called p46 [63] and Nkp44 [82], are associated with the same zeta chain as that of CD3 receptor complexes. Generalized zeta loss, therefore, would be expected to result in the loss of both NK cell and CTL lytic functions.

Correlation between immune dysfunctions, alterations in signal-transducing molecules and stage of disease

Correlation between decreased TCR zeta levels or structurally abnormal TCR/CD3 complexes and cancer stage have been found in colon carcinoma patients [11, 48] and patients with several other types of malignancies [27]. Immunohistochemical studies have also confirmed these findings, demonstrating that, compared with normal gut mucosa, the number of TCR zeta-expressing lymphocytes is decreased in colorectal tumors, being lower in more advanced tumors [57]. In PBL from patients with cervical cancer, a marked decrease in the expression of TCR zeta has been found [41, 58]. In the same study, PBL isolated from patients with CIN to a lesser but significant extent expressed reduced TCR zeta levels when compared with those from healthy donors, raising the possibility that alterations in signal-transducing molecules may occur at relatively early stages in some types of cancer.

More recent examples have been provided from metastatic head and neck cancer where, among 140 patients, those who expressed normal levels of zeta chain had a strong survival advantage over those who had lost zeta expression [69]. In patients with RCC treated with IL-2, only those with complete responses showed recovery of zeta chain expression [67]. Furthermore, patients with pancreatic or colorectal carcinomas who failed to respond to a MUC-1 vaccine, as assessed by DTH responses, had T cells that lacked TCR zeta chains and NF-kB p65 [25]. Following treatment, those individuals who showed recovery of their DTH response, and by in vitro analysis showed an increase in their antigen-specific precursors, showed recovery of their zeta chain and NF-kB p65 levels. Taken together, these findings attest to the potential importance of intact signal transduction mechanisms, and suggest that the levels of signal-transducing and DNA-binding molecules should be measured to monitor the progression of patients in clinical trials.

Transplantable mouse tumors might grow too quickly to reflect the processes that underlie tumor-induced immune suppression in cancer patients

Experimental evaluations of new immunotherapeutic strategies, and the clinical initiatives that arise therefrom, are almost exclusively based on models of transplantable mouse tumors. It has been possible to cure mice bearing established tumors using syngeneic cytokine-secreting tumor cells of the same type, tumor derived antigenic peptides with various types of adjuvants, peptide-pulsed dendritic cells, activated killer cells with or without cytokines, and direct cytokine administration which can be more efficacious if delivered in liposomes. This is in marked contrast to the situation in clinical trials in which only a small proportion of patients have shown responses to similar therapeutic approaches [73].

These apparently contradictory observations between transplantable murine tumor models and cancer patients could be explained in part by the ability of a slowly progressing primary tumor to induce an immune dysfunction as compared to a rapidly growing transplanted murine tumor, which might spare the immune response.

It may therefore be more relevant to study slowly progressing murine tumors as a model for tumor-induced immune suppression. We recently compared primary sarcomas induced by methylcholanthrene (MC) and rapidly growing transplantable MC sarcomas with regard to their ability to generate lymphocytes bearing detectable alterations in their signal-transducing molecule content in B6 mice [38]. Our results demonstrated that mice bearing primary tumors had severely compromised immune responses, with T cells and NK cells showing decreased cytotoxic and proliferative capacity, with reduced levels of Th1 cytokine production and with spontaneous apoptosis of their $CD8^+$ T cells which had markedly reduced levels of the TCR zeta chain. In contrast, the expression of the same molecule and the function of the splenic T cells and NK cells in animals carrying a transplanted, rapidly growing tumor was only marginally affected.

One important implication from this result is that it would be more comparable to the clinical setting to test immunotherapeutic modalities in mice with primary tumors, since their immunological status better reflects that of cancer patients with advanced disease. Research aimed at developing drugs which can counteract suppression of antitumor activity may thus benefit from using primary tumor models rather than transplantable tumors.

Local immune dysfunction in tumor-infiltrating mononuclear cells: possible relationship to systemic late-stage effects in patients with advanced cancer

As discussed above, systemic dysfunctions in cancer patients appear to be nonspecific, to be distinct from clonal anergy or deletion, and to inactivate all subsets of T cells and NK cells [39, 44, 48], indicating that an antigen-independent mechanism is involved. One important point involves the extent to which nonspecific systemic immune dysfunction arises as a consequence of mechanisms that induce local dysfunction of TIL which have been described [52]. Could the systemic defects seen late in advanced cancer represent a ''spreading'' of the same mechanisms initially only operating locally?

The inability of TIL to react with the tumor antigens expressed in situ, despite the observation that $CD8^+$ TIL when cultured in vitro can recognize the autologous tumor, implies the presence of strong immunosuppressive mechanisms which are reversible. Moreover, PBL from patients with late-stage tumors regain their signal-transducing molecules and effector functions when cultured in vitro in the presence of IL-2 [44, 48, 57] or as shown in patients with Hodgkin's disease following stimulation with anti-CD28 and anti-CD3 mAb [70]. Observations such as these argue against an inherent immune defect in lymphocytes from cancer patients. One concern with this interpretation, however, is the possibility that what appears as reversibility might instead be a selective outgrowth of T cells with a normal TCR composition, as the TCR zeta-defective T cells may have a growth disadvantage or be more vulnerable to apoptosis.

Nonetheless, the surgical removal of tumors from patients or animal models has been shown to restore NF-kB and TCR zeta to normal levels [24]. Collectively, this would indicate the presence in the tumor of factors that repress the function of TIL. Soluble factors produced in the tumor, the trafficking of lymphocytes through the tumor, or cells activated locally in the tumor but migrating out into the blood stream, might also affect peripheral T cells. The TCR-zeta levels in T cells and NK cells from noncancerous colorectal tissue from patients with colorectal carcinomas is lower than those in PBL but higher than those in TIL. Thus, a ''gradient'' of TCR zeta as well as of CD16 zeta expression is apparent with samples close to the tumor showing a stronger suppression as compared to those at some distance away from the tumor site [48].

Mechanisms of tumor-induced immune suppression: relationship to alterations in redox status and to apoptosis

Different mechanisms have been suggested to explain late-stage tumor-induced immune dysfunctions and alterations in signal-transducing molecules in PBL and TIL. Chronic stimulation of T cells by antigen in the absence of costimulatory signals leads to the downregulation of the TCR zeta chain and other signal-transducing molecules [12], although this mechanism is not compatible with generalized, polyclonal T cell and NK cell dysfunction. Macrophages with the capacity to suppress immune responses in tumor-bearing hosts have been extensively described [46].

The possibility that monocytes/macrophages can induce immune suppression and alterations in signal-transducing molecules has recently emerged from studies in murine and human systems. Macrophage-derived nitric oxide has been reported to markedly reduce the phosphorylation of JAK3 and STAT5, thus inactivating the ability of IL-2R-positive T cells to proliferate in response to IL-2 [8]. In addition, monocytes recovered by centrifugal elutriation can inhibit in vitro human NK cell-mediated cytotoxicity via secretion of H_2O_2 [31]. The addition of selective scavengers of reactive oxygen species, such as catalase, can counteract the inhibition by oxidant stress on human lymphocyte cytotoxicity and PMA-induced suppression of lymphocyte proliferation [26]. Aoe et al. [4] have reported the ability of tumor-infiltrating macrophages to decrease TCR zeta expression even on freshly isolated normal T cells. This effect is not tumor-specific, but may be a normal consequence of particular activation pathways; thus zymosan/LPS-activated, but not inactivated normal, macrophages also induce TCR zeta downregulation.

However, this phenomenon does occur in cancer patients. Kono et al. [42] have shown that macrophages isolated from metastatic lymph nodes of melanoma patients are able to downregulate TCR zeta levels in autologous PBL. Again, this is not tumor-specific, because LPS-stimulated monocytes from normal PBMC do the same. Because treatment with catalase prevented this, and addition of H_2O_2 duplicated it, it was concluded that reactive oxygen metabolites produced by activated monocytes are responsible for zeta chain downregulation [42]. Otsuji et al. [61] also found that oxidative stress caused by tumor-derived macrophages suppresses zeta chain expression. This could be prevented by treating the macrophages with an antioxidant, as duplicated by treatment of responders with H_2O_2, similar to the phenomenon in cancer patients discussed above. Indeed, pretreatment with the antioxidant enhancer procysteine, which increases intracellular levels of glutathione (GSH), has been found to protect T and NK cells from H_2O_2-induced zeta loss [13].

The possibility of a relationship between cell death and decreased levels of TCR zeta has recently been raised. Tumor-associated lymphocytes obtained from ascitic fluid of women with ovarian carcinoma have decreased levels of cytoplasmic TCR zeta and cell surface-associated TCR eta chains, which is a correlate of decreased T cell receptor function [44]. Subsequently, these authors demonstrated that ovarian carcinomas in situ and in culture express functional Fas ligand (FasL), capable of triggering an intrinsic cell death program in Fas-expressing T cells [68]. The possibility of

a relationship between cell death and TCR zeta loss was examined. Intriguingly, the addition of pan-caspase inhibitors prevented both the in vitro induction of T cell death by ovarian carcinoma cells and losses of TCR zeta and TCR eta chains. These results suggest that TCR zeta chains are involved in the prevention of T cell apoptosis (see below), as has also been observed in mice bearing primary MC tumors [38], in which decreases in TCR zeta content largely parallel decreases in the percentage of splenic T cells and an increased CD4/CD8 ratio. Apoptosis in T cells and NK cells of tumor-bearing mice may therefore be related to their poor functional capacity and also to their decreased expression of signal-transducing molecules.

Recent evidence from a several laboratories suggests that tumor cells bearing FasL actively kill CTL and NK cells [7, 28, 29, 78, 88, 91]. In contrast, the group of Hellstrand has shown that apoptosis is induced in human $CD3^-/CD56^+$ NK cells in vitro after incubation with autologous monocytes producing H_2O_2 [30]. This monocyte-induced NK apoptosis is prevented by the addition of the hydrogen peroxide scavenger catalase. The authors suggested that apoptosis induced by monocyte produced H_2O_2 might in part explain why IL-2 and IFN-α are potent activators of the antitumor activity of NK cells but only rarely reduce the tumor burden in treated patients. Based on the finding that histamine prevents the phagocyte-induced, NK cell-inhibiting signal, they have designed a therapeutic strategy in which histamine and IL-2 or histamine and IFN-α synergize to allow NK cell-mediated killing of human tumor cells [31]. Experimental studies have demonstrated that treatment of tumor-bearing mice with histamine enhances IL-2- and IFN-α-induced destruction of NK cell-sensitive tumor cells in vivo [32].

The results of two pilot trials in metastatic melanoma, initiated by the same group, suggest that the addition of histamine to IL-2 and IFN-α prolongs survival time and induces regression of tumors, such as liver melanoma, which are otherwise considered refractory to immunotherapy [32]. The results of a similar study in advanced acute myelogenous leukemia (AML) suggest that histamine and IL-2 also prolong the remission time in AML patients [32]. In this study it was also observed, that in vivo administration of IL-2 and histamine increases the reduced TCR zeta expression. (J. Sjöberg, in preparation).

It is noteworthy that CD2 might rescue T cells from FasL-induced apoptosis [5], and that ToSo expression might also be protective [37]. Nonetheless, taken together, these findings suggest that apoptosis induced by various mechanisms including FasL/Fas interactions and by free oxygen radicals within the tumor, or possibly even systemically, may severely hamper the immune response in the tumor-bearing individual. The interesting possibility that alterations in signal-transducing molecules may represent "preapoptotic", although reversible, changes in T cells and NK cells induced by the tumor should be investigated further.

Tumor-induced immune suppression may reflect a dysregulation of the immune system also observed in other inflammatory conditions

There is evidence that chronic inflammatory conditions associated with slowly progressing tumors might induce changes similar to those observed in certain infectious or autoimmune inflammatory conditions. As for autoimmunity, a particularly intriguing example of this is rheumatoid arthritis (RA). Similar to TIL, T cells from peripheral blood and synovial fluid of RA patients express increased levels of activation markers, including HLA-DR indicating previous activation [1, 16, 33, 79], but they are functionally impaired. Moreover, poor responses to the polyclonal mitogens PHA and PWM in autologous mixed lymphocyte reactions and following stimulation with antibodies to the CD3 complex of the TCR, as well as decreased production of IL-2 following activation have been described [22, 40, 59, 65]. Taken together, these observations indicate a defect in the signal transduction from the TCR to cytoplasmic elements.

More direct evidence favoring this interpretation has also been reported: crosslinking of CD3 molecules on T cells from RA patients induces a two- to threefold lower Ca^{2+} influx as compared to that in normal controls [53]. As a molecular correlate to this, a decrease in tyrosine phosphorylation of TCR zeta as well as in detectable levels of the TCR zeta protein have been described locally and in the synovial fluid of RA patients [49, 50]. These results suggest that a defect in TCR signaling underlies the hyporesponsiveness of synovial T cells in RA. These alterations in TCR zeta levels have several features in common with the related phenomenon described in cancer patients: the alterations are "global" and observed in all analyzed T cell and NK cell subsets and are reversible following in vitro culture in the presence of IL-2.

Evidence for a possible role of an altered redox state in the hyporesponsiveness of synovial T cells in RA has recently been reported [51]. The hyporesponsiveness of T cells isolated from the synovial fluid of RA patients correlates with a significant decrease in the levels of the intracellular redox-regulating agent GSH. Furthermore, restoration of GSH levels in synovial fluid T cells with N-acetyl-L-cysteine enhances mitogen-induced proliferative responses and IL-2 production. Thus, an altered redox state provides a possible link between the hyporesponsiveness of T cells derived from the rheumatic joint and those from the tumors of cancer-bearing individuals.

Detrimental alterations in signal-transducing molecules may therefore be a common feature in conditions characterized by sustained chronic inflammation. This may also be true for certain chronic viral and bacterial infections, in which similar changes have recently been described. With regard to bacterial infections, the advanced stages of mycobacterial diseases such as leprosy

and tuberculosis, are characterized by a loss of T cell-dependent functions, the basis of which is not well understood. Recently, decreased TCR zeta and p56lck tyrosine kinase expression and loss of nuclear transcription factor NF-kB p65 in T cells of leprosy patients have been described [90]. Of particular interest is that these alterations have been most frequently observed in multibacillary lepromatous leprosy patients, as compared to patients with paucibacillary tuberculoid disease.

Progressive immunodeficiency in cancer patients bears some resemblance to that in HIV-infected patients, including decreased DTH, decreased CTL activity and impaired lymphokine and proliferative responses. Although these patients have a high percentage of HIV-specific $CD8^+$ T lymphocytes, their freshly isolated lymphocytes frequently lack detectable HIV-specific cytotoxicity. However, this effector function becomes readily apparent after overnight culture, a phenomenon similar to the immune paralysis in TIL discussed above. TCR zeta expression levels are downregulated in $CD8^+$ and to a lesser extent in $CD4^+$ T cells from HIV-infected individuals, but these levels are normalized following in vitro culture in IL-2-containing medium [81]. Thus, impaired T cell receptor signaling may help explain why HIV-specific CTL fail to control HIV replication. Chronic macrophage activation and oxidative stress are characteristic features of HIV infection [76], and these features may be a clue to the mechanism responsible and provide yet another similarity between individuals with HIV infection and progressively growing tumors [42, 61].

Concluding remarks

Several important issues regarding tumor-induced immune dysfunctions are stressed above. First, we have indicated fundamental differences between specific immune dysfunctions observed in early stages of tumor growth particularly in the $CD4^+$ subset, which is more related to tolerance, as opposed to that seen locally in the tumor or during later stages of advanced cancer. Although there is evidence that more specific downregulatory mechanisms may also exist in human cancer, as exemplified by the occurrence of T cell regulatory responses in human melanomas [56], most of the available data would argue for a lack of specificity or of selectivity in late-stage tumor-induced immune suppression.

One interesting question, though, is whether the local "ignorance" of tumor antigens observed in human TIL [52] and early among $CD8^+$ T cells during the growth of transplantable tumors [86] has a mechanism similar to the systemic immune dysfunction in late-stage cancer. As discussed above, the systemic immune response in individuals with advanced cancer may also be influenced by mechanisms involving, for example, circulating monocytes which enter tumors and then secrete free oxygen radicals or interact with circulating T cells and NK cells via the Fas/FasL pathway, although direct evidence supporting this is still lacking.

It is also pointed out above that tumor-induced immune suppression and alterations in signal-transducing molecules are not unique to cancer, and that similar phenomena are also found in other chronic inflammatory and infectious conditions, including RA, leprosy and HIV infection. This might arise as a consequence of a negative feedback regulatory mechanism, with a possible physiological role to downregulate T cell and NK cell functions during inflammatory conditions. As there is increasing evidence that monocytes/macrophages play an important role in the control of T cell and NK cell apoptosis, as reviewed above, their role in this regard will be particularly important to evaluate. A central role in these processes is apparently performed by monocytes/macrophages as a result of their ability to induce apoptosis of both T cells and NK cells via elaboration of H_2O_2, and to block their activation via reversible disruption of the JAK3/STAT5 signaling pathway by releasing NO [8]. Does this monocyte-dependent death of activated T cells [85, 87] represent a normal regulatory circuit which has gone awry under these pathological conditions as a result of overactivation during chronic inflammation? Indeed, monocyte-dependent inactivation of tumor-specific CTL and of mitogen-stimulated T cells leading to apoptosis [85] and paralleled by decreased expression of signal-transducing TCR zeta molecules [42] has recently been found.

The data summarized here could explain why various regimens of adoptive or active immunotherapy have failed to generate the desired clinical effects in the majority of treated patients. Vigilant monitoring of the onset of immune abnormalities in cancer patients could be of fundamental importance in predicting which patients will respond to immunotherapy, and in assessing the efficacy of immunotherapy. However, it remains to be shown which immune parameters most accurately predict the capacity of a given patient to respond to one particular treatment modality, and most likely a "battery" of assays should be employed, including DTH to recall antigens, TCR zeta content in T and NK cells, as well as several of the cellular functional assays discussed here.

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