Natural T Cell Immunity against Cancer

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The description of TAAs a decade ago was a groundbreaking step in cancer immunology (1, 2). During the last few years, effective strategies to identify TAAs recognized by specific T cells have been developed and led to the characterization of various families of MHC class I-related TAAs, of which by now more than 60 have been identified (recently summarized in Ref. 3). TAAs can be divided into various groups including differentiation antigens, e.g., melanoma-melanocyte antigens, shared antigens overexpressed on various cancers, such as CEA or telomerase, cancer germ-line antigens, mutated antigens, and viral antigens (reviewed in Ref. 3). These TAAs have facilitated the analysis of T cell responses to tumors and are promising targets for immunotherapeutic strategies. TAA-specific CD8+ T cells represent an important component of the host’s immune response against malignant diseases (4). Clinical studies using immunization with peptides derived from TAAs have shown that high levels of CD8+ T cells with antitumor activity can be raised in cancer-bearing patients (reviewed in Ref. 5). Several recent studies have also shown that induction of specific T cells is associated with clinical effects (6–8).

At the same time, new methods have been developed to analyze T cell responses (9). The high sensitivity of modern single cell assays allows direct analysis of antigen-specific T cells ex vivo, thus eliminating prior in vitro stimulation with cytokines, which could lead to major alterations in T cell state (10). Functional T cell assays, such as the ELISPOT assay and IC-FC, use antigen-specific induction of cytokines to detect specific T cells on a single cell level (11, 12). Multimerized HLA class I molecules carrying a specific epitope-peptide and labeled with a fluorescent marker (tHLA, tetramers) allow the most direct ex vivo staining of specific T cells, but they do not provide data about the functionality of the cells (13–15). In addition, detailed phenotypic analysis of T cells is possible by using flow cytometric methods (IC-FC and IC-FC). Several subpopulations of TAA-specific T cells have been defined using IC-FC or tHLA, and some studies have correlated them with T cell functions. Hamann et al. (16) used CD27 and CD45RA expression to describe three subsets of CD8+ T cells: CD27+CD45RA+ T cells represent naive subpopulations; CD27+CD45RA– T cells represent memory subpopulations; and CD27–CD45RA+ T cells represent effector subpopulations. Sallusto et al. (17) further defined T cells based on the expression of the lymph node-homing chemokine receptor CCR7 as CD45RA+CCR7+ naive T cells, CD45RA–CCR7+ central memory T cells, CD45RA–CCR7– effector memory T cells, and CD45RA+CCR7– differentiated cytolytic effector T cells. A similar distinction of T cell subsets can be made using CD27/CD28 (18). These classifications represent a very helpful tool to further characterize type and function of TAA-specific T cell responses.

Some investigators have performed in vitro restimulation of lymphocytes to increase the frequency of antigen-specific T cells. Although this procedure can result in substantial functional changes of the actual in vivo state of T cells (10), short-time in vitro expansion (not more than 2 weeks) usually does not generate specific T cells from naive precursors, and, thus, comparative quantitative analyses can be performed. Here we consider T cell responses found after short-time in vitro culture as reflecting in vivo stimulation. We labeled these T cell responses as “short-term in vitro stimulation” to distinguish them from T cell responses analyzed directly ex vivo or after longer in vitro.

Abstract

It has long been a matter of debate whether tumors are spontaneously immunogenic in patients. With the availability of sensitive methods, naturally occurring T cells directed against tumor-associated antigens (TAAs) can be frequently detected in cancer patients. In this review, we summarize the current data on T cell responses to TAAs in various malignancies, including melanoma, colorectal cancer, leukemia, and breast cancer. T cell responses against various antigens, including melanoma differentiation antigens, carcinoembryonic antigen, epithelial cell adhesion molecule, her-2/neu, Wilms’ tumor protein, proteinase 3, NY-ESO-1, and survivin, have been reported in a substantial number of patients. In contrast, other TAAs, including most antigens of the MAGE family, do not usually elicit spontaneous T cell responses. A distinction between direct ex vivo T cell responses and in vitro-generated T cell responses is provided because in vitro stimulation results in quantitative and functional changes of T cell responses. The possible role of TAA-specific T cells in immunosurveillance and tumor escape and the implications for immunological treatment strategies are discussed. Naturally occurring T cells against TAAs are a common phenomenon in tumor patients. Understanding the mechanisms and behavior of natural TAA-specific T cells could provide crucial information for rational development of more efficient T cell-directed immunotherapy.

TAAs2 and T Cell Assays

The description of TAAs a decade ago was a groundbreaking step in cancer immunology (1, 2). During the last few years, effective strategies to identify TAAs recognized by specific T cells have been developed and led to the characterization of various families of MHC class I-related TAAs, of which by now more than 60 have been identified (recently summarized in Ref. 3). TAAs can be divided into various groups including differentiation antigens, e.g., melanoma-melanocyte antigens, shared antigens overexpressed on various cancers, such as CEA or telomerase, cancer germ-line antigens, mutated antigens, and viral antigens (reviewed in Ref. 3). These TAAs have facilitated the analysis of T cell responses to tumors and are promising targets for immunotherapeutic strategies. TAA-specific CD8+ T cells represent an important component of the host’s immune response against malignant diseases (4). Clinical studies using immunization with peptides derived from TAAs have shown that high levels of CD8+ T cells with antitumor activity can be raised in cancer-bearing patients (reviewed in Ref. 5). Several recent studies have also shown that induction of specific T cells is associated with clinical effects (6–8).

At the same time, new methods have been developed to analyze T cell responses (9). The high sensitivity of modern single cell assays allows direct analysis of antigen-specific T cells ex vivo, thus eliminating prior in vitro stimulation with cytokines, which could lead to major alterations in T cell state (10). Functional T cell assays, such as the ELISPOT assay and IC-FC, use antigen-specific induction of cytokines to detect specific T cells on a single cell level (11, 12). Multimerized HLA class I molecules carrying a specific epitope-peptide and labeled with a fluorescent marker (tHLA, tetramers) allow the most direct ex vivo staining of specific T cells, but they do not provide data about the functionality of the cells (13–15). In addition, detailed phenotypic analysis of T cells is possible by using flow cytometric methods (IC-FC and tHLA). Several subpopulations of TAA-specific T cells have been defined using IC-FC or tHLA, and some studies have correlated them with T cell functions. Hamann et al. (16) used CD27 and CD45RA expression to describe three subsets of CD8+ T cells: CD27+CD45RA+ T cells represent naive subpopulations; CD27+CD45RA– T cells represent memory subpopulations; and CD27–CD45RA+ T cells represent effector subpopulations. Sallusto et al. (17) further defined T cells based on the expression of the lymph node-homing chemokine receptor CCR7 as CD45RA+CCR7+ naive T cells, CD45RA–CCR7+ central memory T cells, CD45RA–CCR7– effector memory T cells, and CD45RA+CCR7– differentiated cytolytic effector T cells. A similar distinction of T cell subsets can be made using CD27/CD28 (18). These classifications represent a very helpful tool to further characterize type and function of TAA-specific T cell responses.

Some investigators have performed in vitro restimulation of lymphocytes to increase the frequency of antigen-specific T cells. Although this procedure can result in substantial functional changes of the actual in vivo state of T cells (10), short-time in vitro expansion (not more than 2 weeks) usually does not generate specific T cells from naive precursors, and, thus, comparative quantitative analyses can be performed. Here we consider T cell responses found after short-time in vitro culture as reflecting in vivo stimulation. We labeled these T cell responses as “short-term in vitro stimulation” to distinguish them from T cell responses analyzed directly ex vivo or after longer in vitro.

The abbreviations used are: CEA, carcinoembryonic antigen; EpCAM, epithelial cell adhesion molecule; IC-FC, intracellular cytokine flow cytometry; TAA, tumor-associated antigen; tHLA, HLA class I/epitope tetrameric complex; IL, interleukin.
stimulation. Studies in which tumor-directed T cell responses were generated in vitro after long-term culture and repeated stimulation with tumor cells or antigens are not included in this review.

**Natural T Cell Responses**

There is increasing evidence that CD8+ T cells directed against TAAs are spontaneously induced in various malignancies, including melanoma, adenocarcinomas, and leukemias. These studies were made possible by the development of the sensitive and “high-throughput” techniques described above. Mechanisms leading to spontaneous induction of specific T cell responses are not well understood. Here we review the occurrence of TAA-directed CD8+ T cells in patients with various malignancies and in healthy subjects. This report excludes T cell responses induced by antigen-specific immunotherapy. Furthermore, we tried to differentiate between natural T cell responses and T cell responses in patients who had received previous cytokine therapy. However, this is sometimes difficult because, especially in melanoma studies, many patients have received previous adjuvant IFN-α, and the patient characteristics provided are often incomplete. Therefore, it cannot be excluded that the T cell response observed in some patients may have been elicited or altered by previous cytokine therapy.

**Malignant Melanoma**

Cutaneous malignant melanoma is the most extensively investigated human malignancy in tumor immunology. Cutaneous melanoma is considered a highly immunogenic tumor, and several authors have described the presence of T cells with reactivity against TAAs. The most widely studied tumor antigen is the melanoma differentiation antigen melanA/MART-1, against which specific T cell responses were reported in 10–75% of melanoma patients (19–24). Using tetramer staining, T cells responsive against melanA/MART-1 were found in frequencies of up to 0.4% of CD8+ T cells in peripheral blood of melanoma patients (20). Up to 3.5% of melanoma draining lymph node CD8+ cells were identified ex vivo as melanA/MART-1-specific T cells (after short-term in vitro expansion, this percentage increased up to 21%; Ref. 25). Further analyses have shown that peripheral melanA/MART-1-specific T cells are mainly (about two-thirds) CD28+CD45RA

High

or CD45RA

High

CCR7+ representing naive T cells (16, 17, 20, 24). However, one-third of melanA/MART-1-specific T cells are of effector memory type (CD45RA−CCR7−). Interestingly, about 95% of melanA/MART-1-specific T cells at the tumor site represent this effector memory subtype (24).

Less frequently than responses against melanA/MART-1, natural T cell responses are found against two other melanoma differentiation antigens, tyrosinase and gp100 (19, 21–23, 26). Valmori et al. (26) analyzed a tyrosinase

68–376-directed T cell response in a stage IV melanoma patient that reached a frequency of >5% of CD3+CD8+ T cells. IC-FC and tetramer staining showed that most of these naturally occurring TAA-directed T cells were CD45RA+CCR7− granzyme B+, which is characteristic of cytotoxic effector T cells (17, 26, 27). Consistent with their phenotype, tyrosinase-specific T cells were directly lytic ex vivo and specifically recognized tyrosinase-expressing tumor cells (26). In contrast, in another melanoma patient analyzed by Lee et al. (21), tyrosinase

68–376-specific T cells were functionally unresponsive and unable to directly lyse melanoma target cells, although these cells also had many characteristics of effector T cells. Natural T cell responses against gp100 were found against 209-217-2M, a modified peptide, in one patient (22) and against gp100

17–25 after in vitro stimulation in another patient (28).

NY-ESO-1 is a germ-line antigen expressed in cancer, in testis, and, to a lesser degree, in placenta cells. T cell responses against NY-ESO-1 were reported in 3 of 10 (29) and in 7 of 22 melanoma patients (30). NY-ESO-1-reactive T cells in the study of Valmori et al. (29) were CD45RA−CD28+, representing a memory subset of T cells (16). CTLs reactive against another group of cancer germ-line antigens, the MAGE family, are very rarely found, despite extensive studies (19, 22, 23, 31–35). MAGE-A10-encoded nonapeptide

254–262

which is presented by HLA-A2.1, could be an exception. CTL responses to this peptide were detected after short-time in vitro expansion in 8 of 12 patients with a MAGE-A10-expressing melanoma using tetramers of HLA-A2/peptide MAGE-A10

254–262 complexes (36). Interestingly, samples from 3 of 10 patients whose tumors had no detectable MAGE-A10 expression and 2 of 10 healthy donors also contained HLA+CD8+ T cells (36). CAMEL is a translational product of cancer germ-line antigen LAGE-1. The CAMEL-derived peptide

1–11

was detected by specific T cells from peripheral blood of 3 of 33 melanoma patients using tetramers after short-time culture (37). Another group confirmed these results, detecting CAMEL

1–11-directed T cell responses in 3 of 12 melanoma patients by ELISPOT assay after overnight incubation with IL-2 (23). Thus, germ-line antigens show considerable heterogeneity in their immunogenicity. T cell responses are found quite frequently against NY-ESO-1 and MAGE-A10, but not against other MAGE family members. One possible explanation for this may be a difference in the frequency of T cell precursors toward these antigens as suggested by Valmori et al. (36). Recently, T cell responses after short-term in vitro expansion were reported in various malignancies against HLA-A2-binding peptide epitopes derived from survivin, a member of the inhibitor of apoptosis protein gene family. Using ELISPOT assays after in vitro stimulation, specific T cells were detected against survivin peptides Sur1

96–104

, Sur9

65–104

and a modified Sur1 peptide in 2, 3, and 5 of 14 melanoma patients, respectively (38).

However, the T cell responses against known TAAs described above may represent only a minor part of tumor-directed T cell immunity. Letsch et al. (39) have shown that T cells from peripheral blood recognized autologous and/or HLA-matched allogeneic melanoma cell lines in 11 of 19 patients with metastatic melanoma with frequencies up to 0.81% of peripheral blood mononuclear cells. In a further study, T cell responses against autologous melanoma cell lines were found in 5 of 7 patients with T cell frequencies of up to 2.7% of CD8+ T cells (26). Although they showed an overlap of T cell responses against autologous tumor and known antigenic epitopes in one patient, these studies suggest a yet unknown occurrence and magnitude of natural T cell responses, which are most likely directed against a variety of yet-to-be-defined epitopes or TAAs (26). This hypothesis is also suggested by a study showing that
the majority of melanoma-reactive T cell clones do not recognize melanosomal antigens (40).

Most studies have analyzed T cell responses to TAAs in peripheral blood, but this may underestimate the type and magnitude of tumor-specific T cell responses in the tumor and other anatomical compartments. Using intracellular cytokine and tetramer assays, we detected up to 10-fold higher frequencies of tyrosinase- and melanoma-reactive memory CD3+CD8+ T cells in the bone marrow compared with the peripheral blood (41).

Carcinomas

At least 10 TAAs and more than 35 MHC class I antigenic epitopes have been described for colorectal cancer (42). Recently, we demonstrated the existence of naturally occurring T cells that recognized HLA-A2-binding epitopes of TAA EpCAM263–271, her-2/neu654–662, and CEA571–579 in 4, 5, and 6 of 22 HLA-A2+ patients with colorectal cancer, respectively (43). Subsequently, specific T cell responses against at least one of these antigens were observed in 25% of 49 patients (44). Patients with lymph node metastases or distant metastases had specific T cell responses significantly more often. In three patients, a detailed analysis revealed that most of the TAA-reactive CD3+CD8+ T cells detected by IC-FC express CD45RA. CD3+CD8+CD45RA+ T cells immediately producing IFN-γ when exposed to antigen were shown to belong to the effector-type T cell subset that should be able to directly mediate cytolysis (45). In accordance with the above studies, T cell responses against a CEA-derived, modified peptide were observed in approximately one-third of patients with CEA-expressing carcinomas using the IFN-γ ELISPOT assay (46).

Several studies have sought T cell responses against TAAs in breast cancer patients. her-2/neu is a TAA that was identified as a target of cytotoxic T cell lines in patients with breast and ovarian cancers and is also the target for the monoclonal antibody trastuzumab, which is effective in patients with metastatic breast cancer overexpressing her-2/neu (47–49). However, in another recent study (54), T cell responses to proteinase 3 could be detected in patients only after treatment with IFN-α but not after treatment with imatinib (STI571), suggesting that these T cell responses have been induced by treatment with IFN-α. Scheibenbogen et al. (55) studied acute myelogenous leukemia patients for T cell responses to proteinase 3 and the Wilms’ tumor protein (WT-1), a TAA overexpressed in several malignancies. Ex vivo T cell responses to proteinase 3 and WT-1 were found in 2 and 3 of 15 patients with acute myelogenous leukemia using the IFN-γ ELISPOT assay. These results were confirmed by IC-FC showing a TAA-specific population in 2 and 4 of 12 patients with up to 0.7 of CD8+ T cells. TAA-specific T cells were determined as CD45RA+CCR7−granzyme B+ in one patient representing differentiated effector T cells, according to the definition of Sallusto et al. (17). Specific T cell responses were also observed against survivin peptides after in vitro stimulation in patients with chronic lymphatic leukemia (38, 56). Further promising targets for immunotherapy of leukemias (i.e., chronic myelogenous leukemia) are bcr-abl fusion peptides, but no ex vivo T cell response has been detected as of yet, despite the capability of fusion region spanning peptides to bind to HLA molecules and to be immunogenic in vitro (57–59).

Hematological Malignancies

Recently, investigators have started to analyze T cell immunity in leukemia. Molldrem et al. (6) reported a remarkable immunogenicity of the leukemia-associated antigen proteinase 3, which is overexpressed in myeloid leukemias. Using tetramers, they detected a high frequency of T cells specific for this antigen in most chronic myelogenous leukemia patients who achieved complete remission after allogeneic transplantation or IFN-α therapy. However, in another recent study (54), T cell responses to proteinase 3 could be detected in patients only after treatment with IFN-α but not after treatment with imatinib (STI571), suggesting that these T cell responses have been induced by treatment with IFN-α. Scheibenbogen et al. (55) studied acute myelogenous leukemia patients for T cell responses to proteinase 3 and the Wilms’ tumor protein (WT-1), a TAA overexpressed in several malignancies. Ex vivo T cell responses to proteinase 3 and WT-1 were found in 2 and 3 of 15 patients with acute myelogenous leukemia using the IFN-γ ELISPOT assay. These results were confirmed by IC-FC showing a TAA-specific population in 2 and 4 of 12 patients with up to 0.7 of CD8+ T cells. TAA-specific T cells were determined as CD45RA+CCR7−granzyme B+ in one patient representing differentiated effector T cells, according to the definition of Sallusto et al. (17). Specific T cell responses were also observed against survivin peptides after in vitro stimulation in patients with chronic lymphatic leukemia (38, 56). Further promising targets for immunotherapy of leukemias (i.e., chronic myelogenous leukemia) are bcr-abl fusion peptides, but no ex vivo T cell response has been detected as of yet, despite the capability of fusion region spanning peptides to bind to HLA molecules and to be immunogenic in vitro (57–59).

T Cell Responses against Viral (Tumor-Associated) Antigens in Malignant Disease

Several viruses are known to cause malignant transformation of human cells. EBV is associated with Burkitt lymphoma, Hodgkin’s disease, and nasopharyngeal carcinoma. Human papilloma virus infection is strongly associated with cervical cancer. HTLV-1 causes adult T cell leukemia/lymphoma. HHV-8 (Kaposi’s sarcoma-associated herpesvirus) infection can lead to the development of Kaposi’s sarcoma or, less commonly, lymphoma. These viruses encode viral antigens that can be processed by transformed cell and can therefore be considered TAAs. Although natural T cell responses are described against survivin peptides in 3 of 10 breast cancer patients and against NY-ESO-157–165 in 1 breast cancer patient (30). Hoffmann et al. (53) analyzed patients with head and neck cancer using wild-type p53264–272-specific tHLA. Twenty-three of 30 patients had a p53-specific T cell population of up to 0.1% of CD8+ T cells. However, the frequency of p53-specific T cells was higher in patients whose tumors did not accumulate p53, whereas in patients whose tumors accumulated p53, lower frequencies of predominantly naive T cells were found. Also, a proportion of HLA-A2.1+ healthy donors had a low frequency of p53264–272-specific T cells. One possible explanation for these findings is that tumors accumulating p53 have induced apoptosis of p53-specific effector T cells.
antigens derived from these viruses in tumor patients [several EBV antigens, including EBNA3 and LMP2 (60); human papilloma virus, antigens E6 and E7 (61, 62); HHV-8, antigens K12 and K8.1 (63)], their actual role in preventing/controlling malignant development remains to be elucidated. However, the importance of immunological control of virus-associated tumor growth is strongly underlined by the increased development of virus-caused malignancies during suppression or failure of cellular immunity, such as in AIDS (64) or after organ transplants (64).

Private TAAs
Private TAAs are usually restricted to a single patient due to a specific mutation or alteration. In one patient with long-term survival despite incompletely resected squamous cell lung carcinoma, a specific T cell response against a mutated tumor-specific antigen (coded by malic enzyme cDNA) was found by tetramers at a frequency of 0.4% of CD8+ T cells (66). However, it is unclear whether this specific T cell response occurred spontaneously because the patient had received prior vaccination with autologous tumor cells. In another lung cancer (undifferentiated, large cell) patient, specific T cells against an epitope from a mutated α-actinin-4 gene product were detected after short-time culture using tetramers (67). It seems probable that more private TAA-directed T cell responses will be described, especially using new functional genomics approaches (68). Private TAA epitopes including MHC ligands derived from proto-oncogenes or frameshift mutations might become targets for T cell-based therapies in the future with an ongoing individualization of cancer immunotherapy (68).

Healthy Donors
T cell responses against TAAs rarely occur in healthy donors. An exception is melanA/MART-1, against which T cells are present in 8–60% of healthy donors (19, 20, 22). Pittet et al. (20) found melanA/MART-1-directed T cell responses in healthy donors with those found in melanoma patients, data are inconsistent. Chen et al. (69) and Dhodapkar et al. (22) found melanA/MART-1-directed T cell responses more frequently in healthy donors than in melanoma patients, but in most other studies, T cell responses against melanA/MART-1 were more frequently observed in melanoma patients (19, 20, 23, 24, 70). Whereas healthy donors have 95% naive T cells responding against melanA/MART-1, about one-third of peripheral melanA/MART-1-specific T cells in melanoma patients are of the effecter memory type (20, 24). The antigen melanA/MART-1 is unique thus far with its high number of specific T cell precursors in healthy individuals (24). The high frequency of melanA/MART-1-specific precursor T cells could be due to abundant thymic presentation of potentially cross-reactive sequences (71). Interestingly, T cell responses to the melanosomal antigens melanA/MART, gp100, and tyrosinase were found in 35–75% of vitiligo patients, suggesting an important role of these specific T cells in killing melanocyte lineage cells and emphasizing the tenuous balance between immune tolerance and immune defense (72, 73).

With the exception of melanA/MART-1, T cell responses against TAAs are rarely found in healthy individuals. Dhodapkar et al. (22) reported low-frequency responses against tyrosinase and gp100 in 4 and 3 of 12 healthy donors. In contrast, others did not find tyrosinase- or gp100-directed T cell responses in healthy subjects even after overnight incubation with IL-2 (19, 23). Valmori et al. (36) found a T cell response against MAGE-10 in 2 of 12 normal subjects after 2 weeks of in vitro stimulation with peptide, IL-2, and IL-7. Despite extensive investigation in healthy donors, no T cell responses were found against MAGE-1 (19), MAGE-2 (23), MAGE-3 (19, 22), CAMEL (23), CEA (43, 44, 46), Ep-CAM (43, 44), her-2/neu (43, 44, 50, 51), MUC1 (51), proteinase 3, and WT-1 (55). In summary, natural CD8+ T cell responses against melanA/MART-1 in healthy donors have been well demonstrated, but T cell responses against other TAAs are very rare.

Discussion
There is clear evidence that tumor patients are able to generate TAA-specific T cell immunity spontaneously. Whereas the presence of tumor-specific T cells has been shown by many groups and for various tumor types, much less is known about the function of TAA-specific T cells in vivo. Most of the TAA including differentiation, germ-line, and shared overexpressed antigens are not tumor specific but are also expressed at low levels in certain nonmalignant tissues. This should influence the type of T cell response because deletion of functional high-avidity self-reactive T cells in the thymus as well as peripheral deletion or anergy was shown in various animal models (reviewed in Ref. 74). There are a few recent studies analyzing the functional avidity of TAA-specific T cells in patients. In leukemia patients, low-avidity T cells to proteinase 3, which are able to kill leukemia cells, can readily be expanded. However, high-avidity T cells can also be expanded from patients in cytogenetic remission and from healthy subjects, suggesting incomplete self-tolerance to proteinase 3, which is expressed at low levels in normal myeloid cells (75). Similarly, the expansion of high-avidity T cells against the cancer germ-line antigen NY-ESO-1 by peptide vaccination could be demonstrated, although most specific T cells exhibited low avidity (76). In that study, only the high-avidity T cells lysed tumor cells, whereas the low-avidity T cells failed to significantly recognize the tumor targets. On the other hand, it was shown in leukemia patients that high-avidity proteinase 3-specific T cells, although killing leukemia cells more efficiently, underwent apoptosis when exposed to leukemia cells, in contrast to the low-avidity T cells (75). Direct ex vivo cytotoxic function of tetramer-sorted tyrosinase-specific T cells has been demonstrated in one study, whereas in another study, ex vivo sorted tyrosinase-specific T cells failed to lyse tumor cells (21, 26). Several other studies have analyzed the cytotoxic function of short-term in vitro-expanded TAA-specific T cells, showing that the TAA-specific T cells expanded from peripheral blood or lymph nodes can kill tumor cells (25, 29, 36, 77). In two studies, however, TAA-reactive T cells detected ex vivo by IFN-γ-ELISPOT assays could not be expanded in vitro and failed to recognize tumor cell lines (22, 23). Taken together, these data clearly show that the T cell repertoire to self-antigen TAAs has a potential role in antitumor immunity.
Little is known thus far about whether circulating T cells reactive with certain TAAs influence the clinical course of disease. Although data from selected patients suggest a favorable clinical course in patients with natural TAA-directed T cells (26, 55, 66), no study has systematically compared patients with and without TAA-directed T cell responses, however. There is evidence that the presence of intratumoral T cells correlates with improved clinical outcome in various solid tumors (78–80). The assumption that a natural T cell immunity can prevent the development of tumors remains speculative. However, as a matter of fact, tumors can progress despite the existence of TAA-specific T cell responses. Immune escape mechanisms might hamper the effectiveness of natural antitumor immunity. Possible mechanisms include immune tolerance, immune suppression, lack of tumor localization by T cells, Fas/Fas ligand interactions, inadequacy of tumor cells as targets, HLA loss, antigen loss, or anergy induction (reviewed in Ref. 81). An example of antigen loss could be the report of natural MAGE-A10-specific T cells in the blood of three melanoma patients with MAGE-A10-negative tumors (36).

The analysis of natural immune responses in tumor patients has implications for the development of antigen-specific treatment strategies. Because the majority of TAAs described thus far are also expressed in some nonmalignant cells, although mostly at lower levels, the problems of immune tolerance and autoimmunity are important issues to consider when attempting to elicit T cell responses against these antigens. Various animal models have shown that self-reactive T cells are frequently deleted in the thymus or anergized by cells expressing the antigen in the absence of costimulatory signals. The demonstration of functionally active T cell responses against TAAs indicates that the T cell repertoire in adults frequently contains self-tumor antigen-reactive T cells. These specific T cell responses may be activated and enhanced by cancer vaccination or adoptive T cell therapies. One disadvantage of exploiting natural T cell responses for immunotherapy may be that tumor escape variants have been selected already, as outlined above. Of further interest is that despite the presence of T cell responses to TAAs, autoimmunity is rarely observed in patients with cancer. However, caution must be used in drawing conclusions from these data because most studies have not analyzed whether the TAA-specific T cells are able to lyse tumor cells. In one study, the direct ex vivo tumor lytic activity of tyrosinase-specific CD8+ T cells was shown in a melanoma patient who had no signs of skin depigmentation (26).

Most natural T cell responses are reported in patients with advanced disease. This may be simply because most of the patients analyzed had advanced malignant disease. Only a few studies directly compare advanced-stage with early-stage diseases. We found that T cell responses to TAAs occur more frequently in patients with metastatic colorectal cancer than in those with limited disease (43, 44). Also, a higher frequency of autoantibodies against TAAs (Ep-CAM, her-2/neu, tyrosinase, and NY-ESO-1) was reported among patients with metastatic stage of various tumors, including colorectal cancer, breast cancer, and melanoma (82–85). One hypothesis is that TAA-specific T cells in patients with limited disease are at the tumor site, keeping the tumor under control, whereas they are in the periphery in patients with metastatic disease because the tumor does not attract them anymore, due to tumor escape mechanisms. An alternate hypothesis is that the evasion of tumor cells, especially in lymph nodes, is a prerequisite for the induction of TAA-specific T cell responses.

A few reports describe differences in T cell responses against the same TAAs between different tumor entities. One example is breast cancer and colorectal cancer, which share various TAAs (44, 50, 51). Differences in tumor-directed immune responses between these tumors may be explained either by differences in the antigen-presenting capacity of the tumor cells, in the tumor microenvironment, in the local immune system, or in the migratory properties of the TAA-specific T cells (discussed in Ref. 44). There are few studies comparing T cell responses in peripheral blood and bone marrow. Feuerer et al. (51) found functional T cell responses against TAAs in the bone marrow but not in the peripheral blood of breast cancer patients. In melanoma patients, we found TAA-reactive CD3+CD8+ T cell responses in bone marrow in similar or higher frequencies than in peripheral blood, and the subset of TAA-reactive memory T cells was significantly increased in bone marrow (41). Although it is too early to draw general conclusions from these studies, they suggest that bone marrow may be an important compartment for tumor surveillance harboring a tumor-specific memory T cell pool.

Most studies analyzing T cell response to TAAs have emphasized CD8+ T cells thus far. However, CD4+ T cells may play a crucial role in both the induction and activation of TAA-specific memory CD8+ T cells toward cytokotoxic effector T cells (86). CD4+ T cell responses against TAA-derived epitopes have been described for prostate-specific antigen in prostate cancer and for her-2/neu in breast cancer (50, 87). The demonstration of antibody responses to various TAAs, including tyrosinase, NY-ESO-1, Ep-CAM, her-2/neu, and WT-1 (82–85, 88), implies that specific CD4+ T-helper cells to these TAAs should also be present in these patients.

In summary, natural peripheral T cell responses against various TAAs do exist in patients with melanoma, leukemias, and carcinomas. Whether circulating TAA-specific T cells are able to kill tumor cells in vivo remains unclear, as does their effect on the clinical course of disease. Additional studies are necessary for a better understanding of the role of natural T cell responses against TAAs.

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